HISTOPATHOLOGICAL SPECTRUM OF NEUROENDOCRINE TUMORS

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I declare that the thesis entitled **"Histopathological Spectrum of Neuroendocrine Tumors"** has been prepared by me under the guidance of **Dr Harpreet Kaur**, Professor & Head, Department of Pathology, Dayanand Medical College and Hospital, Ludhiana. No part of this thesis has formed the basis for the award of any degree previously.

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"As we express our gratitude, we must never forget that the highest appreciation is not to utter words, but to live by them"

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The mediocre teacher tells
The good teacher explains
The superior teacher demonstrates
The great teacher inspires

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ABSTRACT

Introduction - There has been an alarming increase in occurrence of neuroendocrine neoplasms due to increasing awareness among the physicians and improved diagnostic techniques.

Objectives- To study histopathological spectrum of neuroendocrine tumors.

Materials and methods-The present study was a prospective and retrospective study on histopathological spectrum of neuroendocrine tumors of specimens received in the Department of Pathology, Dayanand Medical College & Hospital, Ludhiana over a period of 5 years (3.5 years retrospectively from 1st July 2015 to 31st December 2018 and 1.5 years prospectively from 1st January 2019 to 30th June 2020). During this period all the specimens were analysed with respect to light microscopic and IHC findings for neuroendocrine neoplasms.

Results- Total of 14,180 malignant specimens were received during the study period, out of which 123 cases were diagnosed as neuroendocrine neoplasms with a mean age of 56 years with a male to female ratio of 1.4:1. Metastatic deposits of neuroendocrine neoplasms were seen in 47.97% cases with the most common site of metastasis as liver followed by lymph node. Maximum cases of neuroendocrine neoplasms were seen in gastrointestinal tract i.e. 56 (45.5%) followed by lung (30.8%). The basic two immunohistochemical markers ie Synaptophysin was positive in 89.38% cases and Chromogranin A was positive in 84.17 % cases. Other neureoendocrine markers such as NSE and CD56 were used. In the gastrointestinal tract maximum cases were seen in small intestine followed by large intestine with most cases between 51 to 60 years with a male to female ratio of 1.9:1. The commonest histological grade of neuroendocrine neoplasms of gastrointestinal tract was Neuroendocrine Tumor Grade 1. Male predominance was seen in lung neuroendocrine neoplasms with maximum cases in 51-60 years. The commonest lung neuroendocrine neoplasms was small cell carcinoma. Hepatopancreatobillary neuroendocrine neoplasms had maximum cases between 61-70 years with a female preponderance. Other organs such as adrenal, breast, prostrate, thyroid etc were also involved by neuroendocrine neoplasms.

Conclusion – Neuroendocrine neoplasms are distinct neoplasms that differ clinically, histopathologically and immunohistochemically from other tumors. So, correlation of histopathological diagnosis with immunohistochemistry is important to give a definitive diagnosis as the prognosis and treatment of these tumors differ from other tumors.

Keywords- Histopathology, immunohistochemistry, neuroendocrine neoplasms.

LIST OF ABBREVIATIONS

CgA	Chromogranin A
CK	Cytokeratin
GEP-NET	Gastropancreatic Neuroendocrine Tumor
GEP-NEN	Gastropancreatic Neuroendocrine Neoplasm
GI-NEN	Gastrointestinal Neuroendocrine Neoplasm
GI tract	Gastrointestinal tract
H&E	Hematoxylin and Eosin
IHC	Immunohistochemistry
LCNEC	Large Cell Neuroendocrine Carcinoma
MANEC	Mixed Adenoneuroendocrine Carcinoma
MEEC	Mixed Exocrine –Endocrine Carcinoma
MiNEN	Mixed Neuroendocrine –Non - Neuroendocrine Neoplasm
NEC	Neuroendocrine Carcinoma
NET,G1	Neuroendocrine Tumor, Grade 1
NET,G2	Neuroendocrine Tumor, Grade 2
NET,G3	Neuroendocrine Tumor, Grade 3
NENs	Neuroendocrine Neoplasms
NSE	Neuron Specific Enolase
PDNEC	Poorly Differentiated Neuroendocrine Carcinoma

PSA	Prostate Specific Antigen
SCNEC	Small Cell Neuroendocrine Carcinoma
SCLC	Small Cell Lung Carcinoma
SYP	Synaptophysin
TLL	Tumor Like Lesions
TTF-1	Thyroid Transcription Factor 1
WDEC	Well Differentiated Endocrine Carcinoma
WDNET	Well Differentiated Neuroendocrine Carcinoma
WHO	World Health Organisation

KEY TO MASTER CHART

C	Cytoplasmic
D	Diffuse
F	Focal
M	Monomorphic
M	Membranous
Neg.	Negative
P	Pleomorphic
Pos.	Positive

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INTRODUCTION

Neuroendocrine neoplasms [NENs] are a heterogenous group of neoplastic proliferations. “Neuroendocrine” term is applied to widely dispersed cells having both “neuro” and “endocrine” properties. “Neuro” property is seen due to presence of dense core granules [DCGs] which are similar to DCGs seen in serotonergic neurons storing monoamines. “Endocrine” property refers to the synthesis and secretion of these monoamines. They share expression of neuroendocrine markers such as Synaptophysin [intracytoplasmic small presynaptic - like vesicles] and Chromogranin A [large dense core hormone granules] but differ in histologic features, hormonal production, proliferation, molecular profile and clinical aggressiveness. The incidence of neuroendrocrine tumors is around 0.5% of all newly diagnosed malignancies. The trend is on the rise, possibly due to increased awareness. [1,2]

Cells of the neuroendocrine system are located in many sites, most common being the gastrointestinal tract [GI tract] and lung. Secretion in blood of bioactive substances, bioamines or peptide hormones can cause clinical syndromes such as the carcinoid syndrome. So, group of NENs with the carcinoid syndrome is denoted as functioning NENs. But, mostly are non-functioning. [3]

Eastern and Western populations have different distribution patterns of NETs. The most common location of NETs is the GI tract. In the United States patients it is small intestine, followed by rectum, colon, stomach and unknown sites according to analysis of the Surveillance Epidemiology End Results database. [4]

An effective framework which is used for the diagnosis of these neoplasms is based on their differentiation degree. Two broad categories are there: well-differentiated neuroendocrine tumors [WDNETs] and poorly differentiated neuroendocrine carcinomas [PDNECs]. [5]

Another category which is represented by neoplasms having a neuroendocrine and a non-neuroendocrine component which can be

adenocarcinoma or squamous cell carcinoma is classified as mixed adenoneuroendocrine carcinomas [MANECs].[5] GI tract and pancreas WDNETs are well-circumscribed lesions having a uniform tan to yellow cut surface. A pushing-type invasion in adjacent tissue is also seen. Histological examination of these neuroendocrine tumors [NETs] shows uniform round to ovoid cells having enlarged nuclei with ample amphophilic or lightly eosinophilic cytoplasm. The cells have plasmacytoid appearance, eccentric nuclei with moderate amount of cytoplasm. [6]

Poorly differentiated neuroendocrine carcinomas [PDNECs] are very aggressive and are poorly circumscribed. They can also show large areas of necrosis and hemorrhage. Frequent metastasis is also seen. Histologically, a solid proliferation of cells, with large nests or sheets having large areas of geographic necrosis is seen. They are divided into small and large cell subtypes depending on the morphological features of cells. [5]

Small cell carcinoma comprises of cells with cell size less than the diameter of 3 small resting lymphocyte. Finely granular nuclear chromatin with absent or faint nucleoli is seen. Frequent necrosis with high mitotic rate is seen. Large cell neuroendocrine carcinoma is present in organoid, nesting, roesttes or trabecular pattern. The cells are large with size more than 3 times the size of resting lymphocytes. Thus, large cell size with low nuclear to cytoplasmic ratio, vesicular nuclei, coarse to fine chromatin with or without frequent nucleoli is seen. High mitotic rate is seen in these tumors. [7]

Mixed adenoneuroendocrine carcinomas [MANECs], are neoplasms having both a neuroendocrine and an epithelial non-neuroendocrine component with each representing at least 30% of the tumor area. [5]

The WHO classification for neuroendocrine neoplasms divides NENs into NET G1, NET G2, NET G3 and NECs, which is based on mitotic counts and the Ki-67 proliferation index, regardless of tumor size, extent or location.

Immunohistochemical markers which are most useful for the demonstration of the neuroendocrine nature of a neoplasm are Chromogranin A and Synaptophysin. Other neuroendocrine markers are neuron specific enolase [NSE], CD56 [Neural Cell Adhesion Molecule- N-CAM], protein gene product 9.5 [PGP 9.5] and CD57. Due to their questionable specificity the routine use of these markers has been discouraged. [5]

Thus, NENs have high incidence and prevalence with heterogeneity in nature. There symptoms can be missed which can lead to late diagnosis. They have a complex pathological classification [grading and staging] but it is the key to understand the tumor behavior. [8]

AIMS AND OBJECTIVES

1. To study histopathological spectrum of neuroendocrine tumors.

REVIEW OF LITERATURE

Neuroendocrine neoplasms [NENs] inspite of their primary site and grade of differentiation are a heterogeneous group of epithelial neoplastic proliferations arising in a number of body organs. These neoplasms share features of endocrine & neural differentiation. Secretory granules & synaptic- like vesicles are present in the cells. Amine and/or peptide hormonal products are also produced by these neoplasms. [5]

In 1870, Rudolf Heidenhain discovered neuroendocrine cells which led to the discovery of neuroendocrine tumors of gastrointestinal tract and pancreas. [9] These endocrine cells in the gastrointestinal tract occupy less than 1% of the mucosa and are distributed at the base of glandular epithelial cells or the surface e.g. gastric pits of stomach, crypts of small intestine and colorectum and have secretory granules which release peptide hormones. In pancreas endocrine cells comprise 1 to 2 % of its volume and are seen as well-circumscribed nests also called as islets of Langerhans. The main pancreatic and larger interlobular ducts also have scattered endocrine cells producing hormones such as insulin, glucagon, somatostatin and pancreatic polypeptide. [4]

The cells can be either epithelial or neuroectodermal in origin. Neuroectodermal cells colonize in various paraganglia and the olfactory membrane. Epithelial cells mainly occur in the mucosa of the gastrointestinal tract, respiratory system, as C-cells in thyroid, parathyroids and pancreatic islets. [2]

Epithelial neuroendocrine neoplasms were described as carcinoid, argentaffinoma, APU Doma, islet cell tumor, medullary thyroid carcinoma, neuroendocrine tumor and neuroendocrine carcinoma whereas the neoplasms with neuroectodermal origin include paragangliomas. [2]

Concept of neuroendocrine tumor was developed in 1900's. Obender for a German pathologist presented an observation on carcinoid tumor in 1907. He recognized the difference between cancerous tumors of the ileocolonic junction and a group of more benign-behaving lesions of the ileum

[carcinoids] and described the tumors that were previously diagnosed as carcinoma as carcinoid. He first described them as benign tumors using the term karzinoide [carcinoma-like] but then revised his observation to malignant tumors which were able to metastasize. Second neuroendocrine tumor which was described was an insulin –producing tumor. [10]

Neuroendocrine cells may secrete bioactive substances, such as bioamines or peptide hormones leading to clinical syndromes. These syndromes are called as carcinoid syndrome when the substances enter in to the blood stream. Thus, the group of neuroendocrine neoplasm which secrete these substances is called as functioning NENs, and which do not secrete are non-functioning. [3]

Carcinoid syndrome is applied to a collection of symptoms which is mediated by various humoral factors. Among the numerous substances involved in the development of carcinoid syndrome, serotonin [5-HT] and metabolites from 5-HT pathway play an important role. Amines, Kallikrein, Pancreatic polypeptide, Bradykinin, Somatostatin, Insulin, S-100 protein, Vasoactive intestinal peptide, Neuropeptide K, Substance P, Neurokinins, ACTH, Gastrin, Growth hormone, Peptide YY, Glucagon, Endorphins, Neurotensin, Chromogranin A and Prostaglandins can cause symptoms such as diarrhoea, bronchospasm, dyspnoea, facial flushing and mesenteric fibrosis. [11]

Neuroendocrine neoplasms occur almost everywhere in the body but are most common in GI tract, lungs and pancreas. Distribution of NETs [Neuroendocrine Tumors] in the GI tract is different between Eastern and Western populations. The commonest location of NETs in GI tract in the United States is small intestine [38%], rectum [34%], colon [16%], stomach [11%] and unknown sites [1%]. In contrast, rectum [48%] is most common site of NETs in the GI tract of patients in Korea, which is followed by the stomach [15%], pancreas [9%], colon [8%], small intestine [8%], liver [7%], appendix [3%] and biliary tract [2%].[4]

There are two different groups of NENs: well-differentiated which are low-proliferating NENs, called neuroendocrine tumors [NETs] and poorly differentiated- which are highly proliferating NENs, called small or large cell neuroendocrine carcinomas [NECs]. [2]

Mostly well differentiated NENs are seen in pituitary, thyroid, parathyroid, thymus, stomach, small bowel, appendix, rectum and pancreas, while poorly differentiated NENs are mostly seen in lung, esophagus, colon, urogenital organs and skin. The mixed neuroendocrin- non-neuroendocrine neoplasms [MiNENs] are commonly seen in the stomach, colon, lung & prostate.

Well differentiated neuroendocrine tumors [WDNET] of the gastrointestinal tract are divided into 3 groups on the basis of their embryologic origin of tubal gut i.e. from foregut, midgut and hindgut. Midgut WDNETs are associated with serotonin secretion and carcinoid syndrome, however hindgut WDNETs are rarely associated with a syndrome. On microscopic examination, the well differentiated neuroendocrine tumors are cellular with uniform round to ovoid cells having enlarged nuclei with ample amphophilic or lightly eosinophilic cytoplasm. Various patterns seen are nests, solid, trabecular, pseudoglandular and gyriform. Rarely, spindled cytoplasmic appearance can be also seen. Poorly differentiated neuroendocrine carcinomas are classified morphologically as either small cell neuroendocrine carcinoma or large cell neuroendocrine carcinoma. Small cell–type have scant cytoplasm with high nuclear/cytoplasmic ratio, fine chromatin and inconspicuous nucleoli and sometimes nuclear molding can be seen. Large cell–type have polygonal cells with moderate amount of cytoplasm, prominent nucleoli and coarse chromatin. Mitoses as well as apoptosis can be appreciated with extensive tumor necrosis. [6]

The prognosis of neuroendocrine neoplasms depends mainly on tumor stage, margin status, WHO grade, histologic differentiation and site of origin. Poorly differentiated neuroendocrine carcinomas have a poor prognosis and a rapid clinical course with rare survival beyond 1 year.

However WDNET has a much better prognosis with a 5-year survival rate. [6]

Management of neuroendocrine neoplasms is done by different therapies which includes mainly surgery. Other types of management such as biotherapy, chemotherapy, peptide receptor radionuclide therapy and tumor embolization can also be used. Several medical therapy options are available for treating the patients having advanced or progressive disease which can lead to significant survival benefit such as stomatastain analogues, interferon – alpha, chemotherapy etc. However, surgery remains the only method for cure and should be considered in all patients if technically feasible. Patients with localized GEP-NET tumors should be offered curative surgical resection of the primary lesion.

Immunohistochemistry with general neuroendocrine markers is mandatory to confirm the neuroendocrine nature of a tumor. The markers include Synaptophysin and Chromogranin A. Synaptophysin is more sensitive which is generally seen in 100% of cases in well-differentiated tumors, while Chromogranin A has more specificity. Typically diffuse, strong staining for one or both of these markers is seen in well differentiated tumors. However, jejunoileal and appendiceal enterochromaffin [EC]- cell neuroendocrine tumors have mostly Chromogranin A positivity, 80% to 90% cases of pancreatic neuroendocrine tumors also show positivity for Chromogranin A and it is mostly weak to negative in rectal and appendiceal tumors. CD56 which is known as neural cell adhesion molecule can also be used as a general neuroendocrine marker but it is discouraged due to lack of specificity. However, poorly differentiated neuroendocrine carcinoma [PDNEC] show Synaptophysin positivity in around 60% of the cases and Chromogranin A in ≤50% of the cases. For a well-differentiated neuroendocrine tumor, keratin immunohistochemistry is highly recommended to confirm the epithelial nature of the tumor. In metastasis of unknown primary, CK7 and CK20 help to assign the site of origin. If epithelial marker is negative in well-differentiated neuroendocrine

neoplasm, the possibility of pheochromocytoma/paraganglioma is considered. [12]

The pattern of Synaptophysin is mostly diffuse and strong, whereas Chromogranin A expression is more variable having diffuse apical and less intense positivity. CD56, CD57 and neuron-specific enolase are not considered specific for neuroendocrine differentiation. Recently, Insulinoma associated protein 1 [INSM1] is a proposed marker of neuroendocrine cell differentiation for tumors in the lung, pancreas and head and neck. Most frequent molecular alteration seen in PDNEC involves the p53 gene, KRAS, p16, Rb1 and cyclin D1 gene. Microsatellite instability [MSI] can also be detected in PDNECs. [6]

WHO CLASSIFICATION OVER THE YEARS FOR GASTROINTESTINAL AND HEPATOPANCREATOBILIARYSYSTEM

1980 WHO Classification- neuroendocrine neoplasms of digestive system were classified as Carcinoid, Mucocarcinoid, Mixed forms carcinoid-adenocarcinoma and Pseudotumor lesions.

2000 WHO Classification of gastropancreatic neuroendocrine tumors –

- Well-differentiated endocrine tumour [WDET]
- Well-differentiated endocrine carcinoma [WDEC]
- Poorly differentiated endocrine carcinoma/small cell carcinoma [PDEC]
- Mixed exocrine-endocrine carcinoma [MEEC]
- Tumour-like lesions [TLL]

2010 WHO Classification of gastropancreatic neuroendocrine tumors-

- NET, G1 [Neuroendocrine Tumor Grade 1]- Ki67, ≤2%, mitotic rate <2/10 HPF
- NET, G2 [Neuroendocrine Tumor Grade 2]- Ki67, 3-20%, mitotic rate 2/10 HPF
- NEC, G3 [Neuroendocrine carcinoma Grade3]- Ki67, >20%, mitotic rate >20/10HPF
- Mixed adenoneuroendocrine carcinoma

2017 WHO Classification of pancreatic neuroendocrine neoplasms

Well-differentiated PanNENs: pancreatic neuroendocrine tumours [PanNETs]:-

- PanNET Grade 1= < 2 Mitotic lndex/10hpf, Ki-67 <3 %
- PanNET Grade 2= 2-20 Mitotic lndex/10hpf, Ki-67 <3-20 %
- PanNET Grade 3 = >20 Mitotic lndex/10hpf, Ki-67 >20 %

Poorly-differentiated PanNENs: pancreatic neuroendocrine carcinoma [PanNECs] which could be large cell or small cell.

- PanNEC Grade 3 = >20 Mitotic lndex/10hpf, Ki-67 >20 %

Mixed neuroendocrine –non-neuroendocrine neoplasm[13]

2019 WHO Classification and grading criteria for neuroendocrine neoplasms including gastrointestinal tract and hepatopancreatobiliary organs:-[14]

Terminology	Differentiation	Grade	Mitotic rate [mitoses/2 mm^2]	Ki-67 index
NET,G1	Well differentiated	Low	<2	<3%
NET,G2		Intermediate	2-20	3-20%
NET,G3		High	>20	>20%
NEC, small cell type[SCNEC]	Poorly differentiated	High	>20	>20%
NEC, large cell type [LCNEC]			>20	>20%
MiNEN	Well or poorly differentiated	Variable	Variable	Variable

Functional classification

GI tract and pancreatic WDNETs can also be classified as functioning or nonfunctioning tumors, if they are associated with a clinical syndrome which is caused by tumor hormone secretion and this functional status of NETs can affect the nomenclature of tumors. Functioning GI tract and pancreatic WDNETs are mostly named after the hormone which is secreted by the tumor resulting in clinical syndrome [e.g. insulinoma]. Functional status can affect patient prognosis e.g. insulinomas generally have an indolent clinical course. [6]

Gastrointestinal Tract

Gastrointestinal neuroendocrine neoplasms [GI-NENs] arise from neuroendocrine cells which are distributed mainly in the mucosa and submucosa of the gastrointestinal tract. The word carcinoid which was used by Obenderfor is a misnomer because inspite the presence of having innocuous- appearing cells with uniform nuclei and few mitoses, these tumors can behave malignantly sometimes with metastasis or with local invasion or recurrence after resection. Terms synonymous with carcinoid such as APUDoma, argentaffinoma, enteroendocrine, tumors of the diffuse endocrine system and argyrophilic cell carcinoma were used in early 1900's.[1]

In 1928, Gosset and Masson characterized these carcinoids as NETs on the basis of their amine uptake and decarboxylation properties. In 1972, Arrigoni et al. characterized "atypical carcinoid" as a histologic variant of typical carcinoid tumor. [1]

In 1980, the World Health Organization [WHO] used the term carcinoid to describe all neuroendocrine tumors [NETs] except pulmonary neuroendocrine tumors which were identified as a separate entity. [1]

The 2000 WHO classification divided the NETs from gastrointestinal tract into well-differentiated endocrine tumors, well-differentiated endocrine carcinomas and poorly differentiated endocrine carcinomas which were based on the degree of differentiation. Both well differentiated and poorly

differentiated endocrine carcinomas were regarded as invasive cancers with the ability to metastasize to distant organs.

In 2000 classification, the term neuroendocrine neoplasm was accepted as a general nomenclature instead of the term carcinoid because carcinoid did not convey the malignant nature of the tumors. [4]

In 2010, a new classification system was announced by the World Health Organization [WHO] for neuroendocrine neoplasms [NENs] of the digestive tract which categorized these tumors as Neuroendocrine Tumor Grade1 [NET-G1], Neuroendocrine Tumor Grade2 [NET-G2], Neuroendocrine carcinoma [NEC] large-cell or small-cell type and Mixed adenoneuroendocrine carcinoma [MANEC]. This classification was made using Ki-67 index and mitotic count. [15]

The 2019 WHO Classification of Digestive System Tumours has updated the classification of neuroendcrine neoplasms of GI tract and hepatopancreatobiliary organs and is now being used as gold standard. It includes the category of “Neuroendocrine Tumor G3” which was absent in the 2010 WHO classification of tumors of digestive system. The 2010 WHO classification referred to neoplasms which were composed of combinations of nonneuroendocrine and neuroendocrine components as “mixed adenoneuroendocrine carcinoma”. But the 2019 WHO classifications has acknowledged that the non-neuroendocrine carcinoma component in these tumors can be squamous as well as adenoacrcinoma, so a new designation to these mixed tumors was given i.e. “mixed neuroendocrine- non-neuroendocrine neoplasms”. The neuroendocrine and non-neuroendocrine component in them must be morphologically distinct, with the minor component representing at least 30% of the tumor. Three short comings which were seen in the 2010 WHO Classification of Tumors of the Digestive System were the G1/G2 Ki-67 hole, well-differentiated neuroendocrine tumor -G3 [WDNET-G3] and mixed tumors having various elements which are dealt in 2019 WHO classification. [12]

Gastrointestinal well differentiated neuroendocrine tumors [WDNET] have significant associations with inherited syndromes most common being Multiple Endocrine Neoplasia 1 [MEN1], Von Hippel-Lindau [VHL] syndrome, Neurofibromatosis 1[NF1] and Tuberous Sclerosis. All of these have an autosomal dominant pattern of inheritance. But these associations are not seen with poorly differentiated neuroendocrine carcinomas. Patients with MEN1 generally have duodenal well differentiated neuroendocrine tumors. The duodenal tumors tend to be gastrin-producing WDNETs which can lead to Zollinger-Ellison syndrome. Patients with MEN1 who have gastrinomas can have enterochromaffin like [ECL] hyperplasia. Somatostatin producing WDNETs of the duodenum can be seen in patients with NF1. [6]

Neuroendocrine neoplasms are divided into well-differentiated and poorly differentiated neoplasms based on the Hematoxylin-Eosin [H&E] appearance of the tumor. The 2019 WHO classification does not have a “moderately differentiated” category.

Grossly the Neuroendocrine tumors [NETs] of gastrointestinal tract are well circumscribed tumors with tan to yellow cut surface, well delinated with a pushing type invasion into the adjacent tissue. However, the neuroendocrine carcinomas are mostly larger and can sometimes show ulcerated masses. Microscopically the NETs belonging to grade 1 and grade 2 are composed of tumor cells which have round or oval nuclei along with “salt and pepper” chromatin and eosinophilic granular cytoplasm. [6]

The G3, WDNET tumors have Ki-67 proliferative index greater than 20% and morphologically is similar to well differentiated NET. In contrast to poorly differentiated neuroendocrine carcinomas [PDNECs], which also have proliferative index greater than 20% but have poorly differentiated morphological features. Most G3, WDNETs have a range of proliferative index in 20% to 55% compared to PDNECs, which generally have a median Ki-67 index of 80%. Loss of TP53 and Rb1 gene is also seen in

PDNEC, whereas it is not seen in G3, WDNET which are associated with DAXX and ATRX loss. [6]

The well-differentiated neuroendocrine neoplasms, including well differentiated neuroendocrine tumor and pheochromocytoma/ paraganglioma, demonstrates “organoid” architecture. However, the latter typically grow in a nested pattern [which is also known as “zellballen” pattern]. In WDNET sometimes one or more architectural patterns, such as nested, trabecular and pseudoglandular can be seen either singly or in combination with the other. Small cell neuroendocrine carcinoma typically demonstrates diffuse architecture, while large cell neuroendocrine carcinoma may have organoid or diffuse pattern. Well-differentiated neoplasms possess low nucleus: cytoplasm [N:C] ratio, necrosis is generally absent and if present tends to be punctuate.[12]

Tumor cells of well differentiated neuroendocrine tumors can also have a plasmacytoid appearance with eccentric nuclei and moderate amount of cytoplasm. The stroma of well differentiated NETs can show fibrosis and in some cases hyalinization can also be seen with a rich vascular network. [6]

On the other hand poorly differentiated tumors such as small cell neuroendocrine carcinoma shows diffuse architecture with high N:C ratio and extensive necrosis. Large cell neuroendocrine carcinomas have large nuclei, irregular nuclear contours, prominent nucleoli, coarse chromatin and extensive areas of necrosis. [12]

Grading is done by using mitotic index and Ki-67 Proliferative index. Number of mitosis is reported as per 2-mm^2 field, which is approximately equivalent to 10 HPF using a 40X objective. So a minimum of 50 HPF should be examined to obtain an accurate number. Most mitotically active areas [i.e. “hotspots”] should be used. [6]

Overestimation of mitotic counts can be made if degenerating/pyknotic nuclei, apoptotic bodies or nuclei of nontumor cells are seen. Whereas the Ki-67 index should be assessed in at least 500 tumor cells with attention

given to hotspots. If the grades based on the mitotic rate and Ki-67 proliferation index show discrepancy then the higher grade has to be assigned. Digital imaging analysis, manual counting or eyeball estimation for Ki-67 can be done. Eyeball estimation method is not reliable and lacks precision, so is not recommended. Lymphocytes, endothelial cells and other stromal cells can prove to be a challenge for quantitation of Ki-67 proliferative index. [6]

Characteristics of each specific organ-

Esophagus:

Neuroendocrine neoplasms of esophagus include well differentiated neuroendocrine tumors [WDNET], poorly differentiated neuroendocrine carcinoma– small cell or large cell type and mixed neuroendocrine –non - neuroendocrine neoplasms. WDNET can be Grade 1, Grade 2, Grade 3. [14]

Esophageal neuroendocrine neoplasms [NEN] commonly occur in the mid to lower segments of the esophagus. Esophageal NETs appear to arise in two ways: as an incidental finding in association with barrett esophagus and adenocarcinoma or as a single large polypoid or nodular tumor. [15]

Majority of patients are diagnosed incidentally by esophagogastroduodenoscopy. Most common symptom is dysphagia followed by abdominal pain, melena and weight loss.[16] The incidence of esophageal neuroendocrine carcinoma [NEC] is approximately 0.4–7.6% of all esophageal carcinoma worldwide.[17]

On gross examination, esophageal NEC may present as a submucosal polypoid, infiltrative growth or as an ulcerating mass on the mucosal surface. Microscopically, the cells are present in nested and trabecular growth pattern with peripheral palisading, rosette formation can also be seen. A high incidence of venous, lymphatic and perineural invasion is also seen. [18]

Immunohistochemical [IHC] staining for several common neuroendocrine markers such as Neuron Specific Enolase [NSE], Chromogranin A [CgA]

and Synaptophysin [Syn] is positive. Thyroid transcriptional factor-1 [TTF-1], which is a kind of nuclear transcriptional factor is highly expressed in epithelial cells of lung and thyroid and is also highly positive in esophageal small cell carcinoma. [17]

Stomach:

Stomach represents about 7% to 8% of all NETs. However, the incidence of gastric NETs is rising probably due to better measures taken for surveillance or because of the widespread use of proton pump inhibitors. [19]

They are graded using 2019 classification of gastropancreatic NENs as Grade 1, Grade 2, Grade 3 NET, NEC and further can be subtyped as Histamine –producing entrerochromaffin –like cell [Type -1, Type-2, Type-3], Somatostatin –producing D-cell NET, Gastrin producing G-cell NET, Serotonin –producing enterchromaffin –cell NET [ECL-NET]. [14]

Gastric NETs are classified into three types:

Type-1 NET which is the most common [70–80%] and is related to chronic atrophic gastritis can occur in the corpus and/or fundus of the stomach as multifocal, small, polypoid tumors. Type-2 NETs is around 5-6% of gastric NETs and are most commonly seen in the corpus and/or fundus of the stomach as multiple mucosal or submucosal tumors. It is associated with MEN-1 and Zollinger–Ellison syndrome [ZES]. Type-3 NETs are around 14–25%. Type-1 and Type-2 NETs are also associated with enterochromaffin- like-cell [ECL-cell] hyperplasia and hypergastrinemia. However, Type-3 NETs are not associated with these conditions. Type-3 NETs seem to be originating from histamine producing cells. [15]

In Type 1 gastric NETs there is a progressive loss of parietal cells and this culminates in achlorhydria, which further leads to D cell suppression and G cell hyperplasia–induced hypergastrinemia. Hypergastrinemia further causes ECL cell hypertrophy and hyperplasia. This can ultimately lead to ECL cell NETs. In type 2 gastric NETs, the hypergastrinemia with resultant excess acid production is mostly due to an ectopic gastrin-producing G

cell neoplasia which is usually located in the duodenum or pancreas. Type 3 gastric NETs have normal gastrin production. [20]

Gastric NETs are positive for Synaptophysin and Chromogranin A. All Enterochromaffin like cells NET are positive for Serotonin, Gherlin, Somatostation & Alpha –HCG.

Diagnosis of type I can be made by upper gastrointestinal [GI] endoscopy and biopsy. Pale, yellowish and transparent blood vessels of the antral mucosa can be seen on endoscopy which is seen in contrast with the smooth and reddish mucosa of normal areas. Neuroendocrine tumors are visualized as small, reddish polyps which are often multiple. Gastric biopsy reveals normal or hypertrophic gastric mucosa in type 2. A single lesion in normal gastric mucosa can be mostly seen in type 3. [21]

Chromogranin A [CgA] which is secreted by ECL cells, under the influence of gastrin, acts as a most useful serum diagnostic marker for NETs with very high sensitivity. [22] Other serum biomarkers except plasma chromogranin A which can be increased are plasma histamine, serotonin and gastrin. Type 1 tumor patients usually do well and type III tumor patients behave more poorly. Thus, type 1 tumors have an excellent long-term prognosis with a normal life expectancy even if metastasis is seen. Type 2 gastric NETs have 5% to 35% spread to regional lymph nodes. Overall, the prognosis of type 2 remains good with a mortality of less than 10%. For most of the type 3 tumors metastatic disease is present in between 50% to 100% of patients. Survival rate is very poor with a mortality rate of 25% to 30%.[19]

NECs are seen as solitary, large, ulcerated tumors which can arise in any part of the stomach. Combined tumors of neuroendocrine carcinoma [NEC] and squamous cell carcinoma of gastric NETs can occur in any part of the stomach as solitary polypoid tumors.These tumors are mostly larger than Type-1 and Type-2 NETs. [15]

Small intestine and ampulla:

Duodenum–

The incidence of Duodenal- NETs [Neuroendocrine Tumors] have increased from 0.027 in 100,000 to 1.1 in 100,000 from 1983 to 2010. [23]

Duodenal well-differentiated neuroendocrine tumors [WDNET] occur as 4 different types: [1] nonfunctioning being the majority [2] ampullary somatostatin expressing [3] gastrinoma [4] gangliocytic paraganglioma. [12]

Non-functioning NENs- These duodenal NENs [Neuroendcorine Neoplasms] are mostly well differentiated and are not associated with an inherited syndrome.

Functioning NENs- Sporadic duodenal NETs which are associated with gastrin are functioning and thus are associated with Zollinger Ellison Syndrome [ZES]. These NETs are therefore called as gastrinoma and around 20% to 30% of these arise with a background of MEN1 and are multicentric. Serotonin-producing NETs causing a carcinoid syndrome are unusual in the duodenum. [24]

Gastrin-producing NET presents mostly within the submucosa as a small polypoid tumor. It commonly occurs in the first part of the duodenum. Histologically these gastrin-producing NETs show trabecular and pseudoglandular patterns. Somatostatin-producing NETs mostly arise is the papilla of Vater. These somatostatin-producing NETs are mostly associated with neurofibromatosis type 1. Psammoma bodies can occasionally be seen. Duodenal NECs [Neuroendcorine Carcinoma] most commonly arise in the papilla of Vater. [15]

Gangliocytic paragangliomas occur mostly in the second portion of the duodenum and ampulla and have triphasic cellular component which include components such as epithelioid neuroendocrine cells, schwannian cells and ganglion cell. The neuroendocrine cells can be seen as cells having an eosinophilic cytoplasm with ovoid nuclei which are arranged in a pseudoglandular pattern or solid nests. [4]

Duodenal NETs are a clinical entity which is different from other small bowel NETs and the majority of the patients present with neoplasms that have a low risk for regional nodal metastasis and the survival of patients is favorable as compared to other small bowel NETs. [23]

Jejunum & Ileum – Neuroendocrine Neoplasms arise from enterochromaffin cells present in small intestine, which constitute approximately 1% of all the mucosal cells. [25]

Comprises half of the small intestinal NETs but are predominantly located in the terminal ileum. These are seen as multiple tumors in up to one-third of the patients. The patients with ileal NETs having metastasis to the liver show signs of carcinoid syndrome, as serotonin bypass the hepatic clearance from the portal circulation. [4]

Common symptoms which are seen in these NETs are abdominal pain, bowel obstruction which can be due to the mechanical effect caused by the primary tumor or due to involvement of mesenteric lymph node by tumor. They can cause the classical carcinoid syndrome which includes diarrhea, palpitations, flushing and bronchospasm which develop due to serotonin-secreting liver metastasis. Due to fibrosis of tricuspid and pulmonary valves, carcinoid heart disease can develop which can further lead to right heart failure in patients with carcinoid syndrome and elevated levels of serotonin. If this serotonin production in carcinoid syndrome continues it can lead to nicotinamide deficiency, rhinorrhea, lacrimation and sometimes diarrhea. [8] NETs can also occur at a rare site as Meckel's diverticulum. These tumours when found incidentally are often small and rarely metastize.

There is a positive immunohistochemical reactivity for CK8, Synaptophysin, Neuron Specific Enolase [NSE] and CD56 in gastrointestinal small-cell NEC. The prognosis for NECs is poor due to the metastatic component of the disease which is seen in most of the patients at the time of diagnosis. [26]

Colon & rectum –

There is a rapid increase in incidence of rectal NETs to 17% of all NETs and now it is approximately equal to that of small bowel NETs. [8] NETs are more frequent in the rectum than the colon. Macroscopically, these rectal NETs are present either as solitary sessile or semi-pedunculated tumors having intact overlying epithelium. Ulceration of the epithelium can be seen in larger neoplasms. Histologically, these rectal NETs show a characteristic trabecular pattern. Majority of colonic NETs are detected in the saecum. Histologically, colonic NETs have a, trabecular, nodular or mixed pattern. In colon, NECs are more common especially in right colon than in the rectum. Large-cell carcinoma followed by small-cell carcinoma is the most common colorectal NEC. [15]

Mostly small rectal NETs are endoscopically detected as submucosal lesions. They cause no hormonal syndrome but can produce glucagon-like peptides and pancreatic polypeptide.

Tumour size can predict malignant behaviour in NETs of the rectum and colon. Regional lymph node involvement likely occurs if the tumor size is larger than 2 cm with invasion into the muscular wall. In contrast to this, rectal NETs which are less than 1 cm in size have a very low risk of lymph node metastasis, however the tumors with size between 1 and 2 cm have a risk of 5% lymph node metastasis. Poorly differentiated tumours have a high rate of metastasis at the time of diagnosis. [24] Best overall survival rate out of gastroenteropancreatic NETs is of rectal NETs. [8]

Immunohistochemically rectal NETs are usually positive for Synaptophysin and negative for Chromogranin A and NETs of the caecal region are positive for serotonin.

Along with positivity of SATB2, other markers which show positivity include OTP, ATRX, clusterin, serotonin, progesterone receptor [PR] and prostatic acid phosphatase [PrAP].[12]

Appendix-

According to WHO 2019, Neuroendocrine neoplasm of appendix are appendiceal epithelial tumors with neuroendocrine differentiation which includes, well differentiated neuroendocrine tumors, poorly differentiated neuroendocrine carcinoma and mixed neuroendocrine–non neuroendocrine neoplasm .[14]

According to WHO 2010 Classification, Tubular Carcinoid was classified as a NET and Goblet Cell Carcinoid was classified as Mixed Adenoneuroendocrine carcinoma.[15]

Appendix Neuroendocrine Neoplasm has an estimated incidence rate of 0.15 to 0.60 per 100,000 per year. On appendectomy majority of the appendiceal well-differentiated neuroendocrine tumors are incidentally discovered. Most of them are Enterochromaffin-cell tumors, 10% to 20% represent L-cell tumors. L- cell tumors demonstrate trabecular architecture and are very small in size .[12]

Tip of the organ is the preferred site of the appendiceal NETs and is mainly observed in women who are 40–50 years. Well differentiated tumors have a size between 1 and 2 cm, can also infiltrate the appendix wall and are composed of serotonin-producing enterochromaffin cells. A size of 2 cm of tumor, with a location at the base of the appendix is associated with deep involvement of the mesoappendix and angioinvasion. These tumors are potentially associated with metastasis. [24]

The risk of lymph node metastasis is 11 % in tumours measuring 1–2 cm with an increases upto 86% in tumours greater than 2 cm. Most tumours are incidentally detected because patients present with symptoms of acute appendicitis.[14] A rare subtype of types of appendiceal NENs is tubular carcinoid [TC] and goblet cell carcinoid [GCC] term is not used now. TC was misdiagnosed as metastatic adenocarcinoma earlier.[15]

Pancreas

Pancreatic neuroendocrine tumours are rare neoplasms of the pancreas which account for less than 5% of all the primary pancreatic malignancies.[27]

In WHO 2017 classification for endocrine organs a classification was devised for neuroendocrine neoplasms of the pancreas [PanNEN] alone and in it the NEC class was by default G3 and the classification emphasized on the association of well-differentiated morphology with the definition of neuroendocrine tumor and the poorly differentiated morphology with neuroendocrine carcinoma. Different molecular pathways were identified for well-differentiated and poorly differentiated neuroendocrine neoplasms, involving MEN1, DAXX and ATRX genes which were observed in NET, G3 but not in NEC and Tp53 and RB gene alterations were seen in NEC but not in NET G3. The concept of Mixed neuroendocrine- non- neuroendocrine neoplasm [MiNEN] was also introduced.[28]

G3, WDNETs that have Ki-67 proliferative indices less than 55% do not respond to platinum-based chemotherapy like PDNEC so it is important to differentiate these two entities.

Care should be taken to distinguish pancreatic well-differentiated neuroendocrine tumors from morphologic mimics such as acinar cell carcinoma which is trypsin positive and solid pseudopapillary neoplasm which shows nuclear B-catenin positivity [12]

Liver, gall bladder & bile ducts

According to WHO 2019 classification liver and gall bladder neuroendocrine tumors are classified as well differentiated neuroendocrine tumors, poorly differentiated neuroendocrine carcinomas and mixed neuroendocrine –non- neuroendocrine neoplasms.[14]

Primary hepatic neuroendocrine tumor [PHNET] is extremely rare with patients often having no clinical symptoms or having some non-specific symptoms e.g. abdominal pain and abdominal mass. But neuroendocrine

neoplasms mostly cause metastasis to the liver and diagnosis of primary hepatic neuroendocrine tumors [PHNET] should be done by excluding extra hepatic NETs. Alpha-fetoprotein levels are also not high.[29]

Other NETs produce biologically active polypeptides or amines, but PHNET does not produce as a result of which there is no carcinoid syndrome.[30] 50–60% of patients with gastroenteropancreatic NETs [GEPNETs] mostly present with regional or distant metastasis and liver is the most common site of metastasis in 85% of patients .

Lung

In 1970, lung neuroendocrine tumors were classified into: Typical [TC] and Atypical [AC] carcinoid and small cell lung carcinoma [SCLC]. The fourth edition of the WHO classification re-defined large cell neuroendocrine carcinoma [LCNEC] as a neuroendocrine neoplasm. Thus currently neuroendocrine tumors of the lung are classified into four main categories: TC, AC, LCNEC, and SCLC. [31]

WHO 2015 classification of neuroendocrine lung tumors is-

- Carcinoid =0-1 Mitoses/2 mm^2, Ki-67 – upto 5%
- Atypical carcinoid=2-10 Mitoses/2 mm^2, Ki-67 – upto 20%
- Small cell Lung carcinoma= >10 Mitoses/2 mm^2, Ki-67- 50-100%
- Large cell Neuroendocrine carcinoma = >10 Mitoses/2 mm^2, Ki-67- 40-80%.[7]

An increase in incidence of pulmonary carcinoids, with a rate of 1.4 to 2.0 per 100,000 has been seen in more recent times. They are defined as malignant neuroendocrine neoplasms which are larger than 5.0 mm and are not associated with cigarette smoking with almost equal male to female incidence and are mostly seen in younger patients as compared to higher-grade neuroendocrine neoplasms. Carcinoids are often centrally located however around 30% are also found in peripheral locations.

Carcinoids are usually solitary lesions with various growth patterns such as solid, nests, trabecular, ribbon like or rosette. The most consistent histologic finding which is seen is uniformity of the cell nucleus. Chromatin

in the cells is described as salt and pepper i.e. stippling of chromatin is seen. Tumor in the carcinoid group can be typical and atypical carcinoid. It is based on 2 features: necrosis and mitotic activity. Typical carcinoids have fewer than 2 mitoses per 2 mm^2 [10 high-powered fields [HPF] and lack necrosis. Features like calcification, stromal hyalinization & stromal amyloid can also be seen in carcinoid. Several carcinoid mimickers are there such as low grade mucoepidermoid carcinoma which show morphological similarities with an overlapping age group. Presence of mucous-producing cells is seen in mucoepidermoid carcinoma, but the predominance of intermediate cells is a pitfall due to uniformity of the cells. Metastatic carcinomas can also grow in solid patterns with bland nuclei. [32]

Atypical carcinoids have 2–10 mitoses per 2 mm^2 [ten high power fields] and/or necrosis in addition to a neuroendocrine morphology which is similar to TCs.

The impact on prognosis of diagnosis of typical and atypical carcinoid is significant. In typical carcinoids, lymph node metastasis e.g. N1 stations may not have an impact on overall survival and sometimes not require adjuvant therapy. However, atypical carcinoid may require adjuvant therapy even without having nodal disease, and is also sometimes influenced by tumor size status with consideration of complete lobectomy and the five-year survival for typical carcinoids mostly exceeds 90%, however that of atypical carcinoids is around 60%.[32]

SCLC likely occurs in males and there is seen a strong relationship with smoking. It usually shows a diffuse growth pattern. Morphology, such as organoid, rosette and trabecular patterns are not seen. [33] The cells of small cell carcinoma have high nuclear-to-cytoplasmic ratio with scant cytoplasm leading to nuclear molding. Histologically, SCLC have a high mitotic rate i.e. more than ten mitoses per 2 mm^2 and frequent necrosis is seen. The size of these cells is less than the diameter of three small lymphocytes. It is a high-grade neuroendocrine tumor with patients having

a very poor prognosis often with a 5-years overall survival rate of about 16.4%. [31]

High nuclear-to-cytoplasmic ratio with scant cytoplasm cells are seen in small cell carcinoma thus leading to nuclear molding. The cells are very fragile thus frequent crush artifact can be seen. Classic salt pepper chromatin with fine chromatin without nucleoli is seen and necrosis is present both in small-cell and large cell NECs. [32]

When SCLC and non-small cell carcinomas, for e.g. adenocarcinoma, squamous cell, large cell neuroendocrine carcinoma, spindle cell carcinoma, and giant cell carcinoma, are combined, those tumors are called combined SCLC.

LCNECs are typically seen in the periphery, are lobulated, and sometimes speculated masses can be seen. LCNEC have large cells more than the size of 3 lymphocytes having visible cytoplasm and identifiable nucleoli. [32]

Patients are predominantly males with association with smoking and are older in age. LCNEC have high Ki-67 index. Genetic alterations are more frequent in SCLC >LCNEC than other neuroendocrine tumors of lung according to microsatellite marker analyses. [31]

Small-cell carcinomas sometimes have large-cell neuroendocrine carcinoma components and such combinations are called as combined small-cell carcinoma. Clinical impact on lung cancer treatment is seen because small-cell carcinoma remains a subtype having nonsurgical therapeutic approach. Primary surgical approach is followed in large cell neuroendocrine carcinoma. Small-cell carcinomas mostly present as advanced disease, whereas large-cell neuroendocrine carcinomas can be detected at a very early stage. Also paraneoplastic syndromes are associated with small-cell carcinomas which are rarely seen in LCNEC. [32]

Other tumors which are primitive neuroectodermal tumor, lymphoma, desmoplastic small round cell tumor, mesenchymal chondrosarcoma and small-cell osteosarcoma should be kept in differential diagnosis of small cell neuroendocrine carcinoma.

For large cell neuroendocrine carcinomas the differential diagnosis includes small cell carcinoma, nonkeratinizing squamous cell carcinomas, basaloid carcinoma and solid pattern adenocarcinoma. Cytokeratin along with neuroendocrine markers confirm LCNEC and p40 will eliminate squamous cell carcinomas. Cytokeratin immunoreactivity is mostly seen in carcinoid tumors with positivity of markers such as Synaptophysin, Chromogranin A and CD56 which are positive. Chromogranin A is the most specific.

Immunohistochemically, the tumor cells of lung neuroendocrine carcinoma are positive for neuroendocrine markers [Chromogranin A, Synaptophysin, CD56] and thyroid transcription factor-1 [TTF-1]. TTF-1 is usually not present in lung carcinoids but is approximately seen in 80-90% of SCLCs and 50-60 % of large cell neuroendocrine carcinomas. [33]

Immunohistochemistry for Cytokeratin and Synaptophysin is often dot like in small-cell carcinoma, whereas it is membranous for cytokeratin or diffuse cytoplasmic synaptophysin positivity in cases of LCNEC.

Breast

In 2003 WHO for breast tumors recognized neuroendocrine tumors as a distinct histological entity, having the same morphological characteristics as neuroendocrine tumors of the gastrointestinal tract, pancreas or lung. 50% of the tumor cells show positivity by a neuroendocrine marker such as chromogranin or synaptophysin. This classification excludes mammary carcinomas having focal neuroendocrine differentiation which is defined as scattered tumor cells which stain positive with neuroendocrine markers. Expression of Her2 is generally absent whereas a strong expression of estrogen and progesterone is seen. [34]

In 2012 WHO classification of tumors of breast, neuroendocrine tumors were classified into two main categories -1] Neuroendocrine tumors–Well differentiated which included low and intermediate grade tumors, 2] Neuroendocrine carcinomas, poorly differentiated / small cell carcinoma. Large cell neuroendocrine carcinoma was not included. Neuroendocrine

carcinomas was included in the category of carcinomas with neuroendocrine features. A third category was also included which consisted of a subset of breast carcinomas with neuroendocrine differentiation determined by histochemical and immunohistochemical stains. This category included invasive duct carcinoma of no special type, solid papillary carcinomas, hypercellular subtype of mucinous carcinoma. [35]

The 2019 WHO Breast classified neuroendocrine neoplasms as neuroendocrine tumors and neuroendocrine carcinomas –small cell /large cell type. If the small cell carcinoma makes upto 10-90% of the tumor area the terminology for mixed invasive and small cell carcinoma can be used, if <10% pattern is there of neuroendocrine neoplasms then it should be classified as invasive NST or other types and if > 90% neuroendocrine neoplasm pattern is there then it should be classified as neuroendocrine tumor or neuroendocrine carcinoma. [35]

Primary breast neuroendocrine tumors are very rare histological type and represent less than 0.1% of all breast cancers.These tumors are mainly observed in white women around 60 to 70 years. Nospecific clinical signs are related to these tumors.

Female genital tract

Ovary-

Neuroendocrine tumours are uncommon or mostly rare at all sites in the female genital tract. Most commonly seen in the ovary followed by uterine cervix. In the 2014 WHO Classification of ovarian tumours, no separate category of neuroendocrine neoplasms, unlike at other sites in the female genital tract.

The term carcinoid tumour is still used and is included in the category of “Monodermal teratomas and somatic- type tumours arising from a dermoid cyst” in the 2014 WHO Classification. Well-differentiated neuroendocrine tumour grade 1 is also listed as a synonym of carcinoid tumor. The most common primary neuroendocrine neoplasm are ovarian carcinoid tumours

in the female genital tract and almost all arise within teratomas, especially dermoid cysts. Patients with primary ovarian carcinoids rarely have the carcinoid syndrome. It is mostly unilateral and is an incidental microscopic finding. Morphologically, four main variants of primary ovarian carcinoid tumour comprising of insular [most common], trabecular, strumal and mucinous [goblet cell] are seen. The latter two i.e.strumal and mucinous are listed as variants of carcinoid tumour in WHO 2014. Insular carcinoids morphologically resemble midgut carcinoids with the tumour cells having polygonal, round or ovoid nuclei with “salt and pepper” chromatin and abundant cytoplasm. Trabecular carcinoids are less common than insular. Strumal carcinoids are mainly composed of a mixture of carcinoid elements and strumaovarii. The rarest primary ovarian carcinoid tumours mucinous [goblet cell] carcinoid and is composed of small glands or acini which are lined by columnar or cuboidal epithelium with abundant intracytoplasmic mucin and variable numbers of goblet cells.[36]

“Small-cell carcinoma of pulmonary type” is a category of miscellaneous ovarian tumours which is morphologically similar to pulmonary small-cell carcinoma and thus the term small-cell carcinoma of neuroendocrine type is also listed as a synonym.

Uterus-

The endometrial neuroendocrine tumours in 2014 WHO Classification includes low-grade neuroendocrine tumour [carcinoid tumour] and high-grade neuroendocrine carcinoma [small-cell and large-cell neuroendocrine carcinoma]. Both low-grade neuroendocrine tumours and high-grade neuroendocrine carcinomas of the endometrium are uncommon tumours.

Cervix-

NETs of the uterine cervix are very rare having a poor clinical outcome. They have early lymph node involvement. Cervical neuroendocrine neoplasms are categorized into low-grade neuroendocrine tumour and high-grade neuroendocrine carcinoma. Cervical high grade neuroendocrine carcinomas are mostly HPV-associated neoplasms.

Occasionally some neoplasms are composed of a mixture of small-cell and large-cell neuroendocrine carcinoma. In small-cell neuroendocrine carcinoma a monotonous population of cells with ovoid or spindled hyperchromatic nuclei exhibiting moulding and scanty cytoplasm is seen. Abundant mitotic and apoptotic activity is there. Predominantly diffuse growth is seen but sometimes nests, trabeculae, pseudoglandular and rosette- like structures are also present. Large-cell neuroendocrine carcinoma is characterised by large cells with a low nuclear to cytoplasmic ratio, nuclei having coarse chromatin with prominent nucleoli and high mitotic activity. Insular, nested, trabecular, pseudoglandular and solid patterns are often present along with extensive geographical necrosis. [36]

Vulva &Vagina-

High-grade neuroendocrine carcinoma of the vulva or vagina is essentially a diagnosis of exclusion, as the histological and immunophenotypic features are identical to those of neuroendocrine carcinomas arising at other sites.

Male genital tract

Prostrate-

The 2016 World Health Organization classified prostatic neuroendocrine tumors into 5 groups:

1) Adenocarcinoma with Neuroendocrine [NE] differentiation refers to the cases having characterstics of typical adenocarcinoma acinar or ductal type with focal NE cells which are appreciable by the help of IHC stains. Number of NE cells varies but is generally no more than 1% of the entire tumor cell population.

2) Adenocarcinoma with Paneth cell–like NE differentiation is a typical adenocarcinoma of the prostate with varying proportions of cells with prominent cytoplasmic granules. These cells express NE markers.

3) Carcinoid tumor is a well-differentiated NE tumor with polygonal or spindle-shaped cells having small uniform nuclei and an insular, nested,

glandular or mixed architecture. These cells are positive for NE markers [Synaptophysin, Chromogranin A and CD56] and negative for PSA.

4) Small cell NE carcinoma grows as solid sheets, cords and single cells. They are generally negative for PSA and expresses CD44 in a distinct membranous pattern.

5) Large cell NE carcinoma occur as solid sheets, ribbons or nests with focal necrosis in the center with areas of peripheral palisading.[37]

Testis-

Carcinoids represent the only type of neuroendocrine neoplasm that is mentioned in the current WHO classification for testicular tumour.

Head & Neck

Neuroendocrine tumors of the head and neck are very rare. These heterogeneous group of neoplasms are categorized by the 2017 WHO Classification of Head and Neck Tumors as well-differentiated [WD-NEC], moderately-differentiated [MD-NEC] and poorly-differentiated [PD-NEC] with small cell and large cell types of hypopharynx, larynx, trachea and parapharyngeal space. The use of the terms “carcinoid” and “atypical carcinoid” as diagnostic categories are not used. [38]

Histologically, the cells are small with hyperchromatic nuclei and scant cytoplasm. They generally have areas of necrosis and high mitotic activity.

Tumors of C-cells [parafollicular cells] i.e. majority of thyroid NENs which are known as “medullary thyroid carcinoma” [MTC] overall represents 3-5% of all thyroid carcinomas. Mostly develop in patients with MEN2 syndromes. They are mostly well- differentiated NETs and show expression of TTF-1 & calcitonin with PDNEC representing less than 1% of MTCs. A small cell variant is also included which is to be distinguished from metastatsis of small cell carcinoma to the thyroid gland. Serum calcitonin and carcinoembryonic antigen levels are to be kept in consideration for medullary thyroid carcinoma.[39]

Others

Pheochromocytomas are rare neuroendocrine tumors of the adrenal medulla which produce catecholamine & store excessive amounts of norepinephrine and epinephrine, which if released, during anesthetic induction or surgical removal, can produce cardiovascular complications. When this tumor arises outside the adrenal medulla, it is known as paraganglioma.[40]

Pheochromocytomas and paragangliomas have the classic zell ballen morphology which is characterized by nests of uniform polygonal chromaffin/chief cells surrounded by supporting cells i.e. sustentacular cells. Chief cells express Synaptophysin, Chromogranin A and CD56 [cluster of differentiation 56) but epithelial markers are not expressed. If Chromogranin A is not expressed, then the diagnosis of Pheochromocytoma should be re-considered. Sustentacular cells are highlighted by S100 protein.[41]

The prototypical primary cutaneous NEN is Merkel cell carcinoma with etiology related to the clonal integration of the Merkel cell polyoma virus and/or ultraviolet radiation.

Immunohistochemistry

The most specific immunohistochemical markers for neuroendocrine neoplasms are Chromogranin A [CgA] and Synaptophysin.

CgA is an acidic glycoprotein having diffuse cytoplasmic positivity being expressed in well to moderately differentiated NENs and mostly is focally positive in poorly differentiated neuroendocrine carcinoma. It can have limited sensitivity with some tumors eg hindgut carcinoids

CgA has a high sensitivity ie around 73% and specificity around 95% for the diagnosis of NETs with variation of sensitivity according to the site. Higher sensitivity is seen in gastrinomas and gastric NETs but has a lower sensitivity in cases of pancreatic NETs.[8]

Synaptophysin is a membrane glycoprotein and has a diffuse cytoplasmic immunostaining.

The glycoprotein is isolated from nervous tissue which are present in presynaptic vesicles of almost all nerve cells. In adrenal medulla, cells of pancreatic islets and neuroendocrine tumour Synaptophysin is expressed. It is one of the most specific markers of tumors originating from neuroendocrine cells, with a much higher sensitivity than Chromogranin A and NSE, mostly for diagnosis of lung tumours.[42]

Generally diffuse immunoreactivity is seen with Synaptophysin both in WD-NETs and PD-NECs, whereas Chromogranin A expression may not be present in PD-NECs or if present, it is focal. Pattern of immunoreactivity may show perinuclear dot-like staining.[5]

Neural cell-adhesion molecule which is also called as CD56 is a membrane-bound glycoprotein and it is involved in interactions between neuron-neuron and nerve-muscle. It is sensitive, but it is not a highly specific marker for these neoplasms. [3]

Neuron-specific enolase [NSE] is also expressed in NETs, but is not considered specific enough to show neuroendocrine differentiation when it is used in isolation.

Insulinoma associated protein 1 is proposed as a general marker of neuroendocrine cell differentiation. It is a nuclear transcription factor which is a sensitive marker of neuroendocrine differentiation.

Around 10 to 20% of WD-NET can present as metastasis especially from jejunoileum, pancreas, lung and other sites.[12]

In cases of metastasis, site of origin is to be determined as it has implications on treatment and prognosis. If clinical history, physical examination, radiographic studies do not help in determining the site of origin then immunohistochemical markers of site of origin such as CDX2, TTF-1, PAX8, SATB2, PSA can be used .

Broad-spectrum keratin immunohistochemistry in cases of well-differentiated neuroendocrine tumor is recommended in order to confirm its epithelial nature. In cases of metastasis of unknown primary, CK7 and CK20 should be performed to help assign site of origin.[12]

“First round” emphasizes should be given on identifying tumors of jejunoileal and pancreaticobillary origin so CDX2 is used which is expressed in 90% of jejunoileal tumors and 15% of pancreatic tumors. Islet 1 is the most sensitive pancreatic marker. SATB2 can be used for lower GI well-differentiated neuroendocrine tumors. For WD-NET, CDX2, TTF-1, PR, SATB2 should be performed assuming positivity for broad spectrum epithelial marker and Synaptophysin and Chromogranin A.

If CDX2 is positive with all other markers negative, then midgut can be the site of metastasis. If PR, PAX 8 is positive, SATB2 negative then pancreas should be considered and if TTF-1 is positive then lung should be considered and SATB2 positivity is seen in rectum and appendix.

P53 and Rb for distinction between well-differentiated neuroendocrine tumor G3 and poorly differentiated neuroendocrine carcinoma is required. P53 wild type and RB intact is seen in neuroendocrine tumor grade 3 and P53 mutant and RB Null is seen in poorly differentiated neuroendocrine carcinomas.[12]

Ki-67 labeling index is a proliferative marker for NENs and is independently correlated with survival and represents the most reliable prognostic factor for neuroendocrine neoplasms.[3]

Fine-needle aspiration is an important modality which can be used for the primary diagnosis and staging of neuroendocrine neoplasms.[43]

Thus, neuroendocrine tumors are a heterogeneous population of cancer subtypes with morphologies ranging from neuroendocrine neoplasms to poorly differentiated carcinoma including small-cell and large-cell NECs and express neuroendocrine markers, such as CgA and Synaptophysin. [1]

A large autopsy series from Sweden in 1975 which was held between year 1959 and 1969 reported an annual incidence rate of 8.4/100,000 for neuroendocrine neoplasms which was much higher than the rate reported in clinical series held in the same period. This discrepancy emphasized the fact that many neuroendocrine neoplasms can remain asymptomatic throughout life without showing any clinical symptom [44]

Halla Skuladottir et al in 2002 did an analysis in Denmark on patients who were diagnosed with bronchial neuroendocrine tumor. The study found that the incidence of neuroendocrine tumors has increased by two fold among men and by three fold in women, thus concluding that the incidence of neuroendocrine tumors is increasing.[45]

A study of 15 cases was done in 2010 of primary esophageal small cell carcinoma which were retrospectively analyzed for clinicopathological and immunohistochemical features in a hospital of China from 1990 to 2004, in order to find suitable diagnostic markers and applicable therapies for the carcinoma. The records which were included were presenting symptoms of the patients, demographics, diagnostic method, histopathology, follow–up and therapy. The study suggested that PESCC was a rare and aggressive tumor with a higher proportion of positive labeling of NSE, Synaptophysin, Cytokeratin, CD56 & TTF-1.[46]

A retrospective study at M. D. Anderson Cancer Center done in 2010 collected the data between 1990 and 2005 on 80 patients diagnosed with carcinoid tumors of lung. The cases were reviewed and features taken into consideration were chromatin pattern, presence of nucleolus, nuclear pleomorphism, infiltrative growth, blood or lymphatic vessel invasion and necrotic patterns. Sixty eight cases were diagnosed as typical carcinoids and 12 with atypical carcinoids. Criteria for distinguishing atypical carcinoids from typical carcinoids included the presence of comedo- like necrosis and/or an overall mean number of mitoses which was ≥0.2/HPF. [47]

A study done by kendall J keck et al at University of Iowa NET Clinic between 1999 and 2016 included 197 patients with metastasis. Primaries were identified preoperatively in 168, by operative exploration in 7 cases and in 22 patients it was not found. The primary neuroendocrine tumor was mostly found as small bowel followed by pancreas stomach, duodenum and rectum.[48]

Francisco et al in 2017 analysed retrospectively patients of esophageal neoplasm from 2008 to 2016. Fourteen patients were identified as neuroendocrine tumors. Amongst which were 11 males and 3 females with the mean age as 67.3 years. Small cell carcinoma was seen in 10 patients and large cell neuroendocrine carcinoma in 3 and 1 was carcinoid. The conclusion of the study was that neuroendocrine esophageal tumors are extremely rare and affected men mainly.[49]

Meng Zhang et al did a retrospective study in 2017. Clinicopathological features of gastroenteropancreatic neuroendocrine neoplasms were studied. Four hundred forty patients were included and primary sites of neuroendocrine neoplasms came out to be stomach [24.3%], rectum [24.1%], pancreas [20.5%], esophagus [12.3%], unknown primary origin [8.0%] and duodenum [6.1%] with immunohistochemical positivity rate of Synaptophysin as 97.7% and of Chromogranin A as 48.7%. Thus, concluding that gastropancreatic neuroendocrine neoplasms may originate from any part of the digestive system.[50]

In another study done by Felipe Silveira et al in 2017 comparison of the frequency of neuroendocrine tumors according to age, location, gender, degree of differentiation was done in 250 patients, lung came out to be the most frequent site followed by stomach and liver metastasis was found in 44 cases thus concluding the fact that neuroendocrine neoplasm can affect any organ.[51]

A study done by Fatma ŞENEL et al in 2018 included a total of 13,863 patients retrospectively. All of them underwent histopathological examination of appendectomy specimen between January 2007 and March 2017. In the study NET [Neuroendocrine tumor] was detected in 29 cases [0.2%] of 13,863 appendectomy specimens. Of these 29 cases, 26 were carcinoid tumors and 3 were goblet cell carcinoid tumors [GCCTs] with the mean age as 37 years and male prepondrance. Thus according to the study NETs of the appendix and GCCT cases were slightly higher in males and advanced age groups. [52]

According to the study done by Rashi garg et al in 2018 on 42 cases of neuroendocrine tumours of lungs excluding small cell carcinoma between a span of january 1999 to august 2014 the Haematoxylin & Eosin stained sections, immunohistochemistry for neuroendocrine markers and Ki-67 were studied. Based on WHO criteria, cases were classified as typical carcinoids [83.3%], atypical carcinoids [12%] and large cell neuroendocrine carcinomas [4.7%] with the Ki-67 index ranging between 1%-10%, 10%-30%, 35%-50% in typical carcinoid, atypical carcinoid and large cell neuroendocrine carcinoma respectively. Tumours with carcinoid morphology, <2mitoses per $2mm^2$ with no necrosis were classified as typical carcinoids and those with carcinoid morphology but 2-10 mitoses per $2mm^2$ or necrosis were classified as atypical carcinoid. Similarly, the tumours with neuroendocrine morphology, positive immunohistochemical staining for one or more neuroendocrine markers and high mitotic rate: ≥11 per $2mm^2$ with, large zone of necrosis, large-cell size, low nuclear to cytoplasmic ratio, fine chromatin, with presence or absence of frequent nucleoli were classified as large cell neuroendocrine carcinoma. Thus, concluding that morphological features are the gold standard in subtyping of neuroendocrine neoplasms along with Ki-67 acting as a meaningful marker for sub-categorization of lung neuroendocrine neoplasms. [53]

A retrospective study done in 2018 evaluated 35 patients having primary lung NETs which were being treated at National Cancer Institute of Egypt between year 2010 and 2014 by Magdy Saber et al. Study showed a male predominmance with the mean age of patients as 53 ± 11.2. SCLC was the most prevalent histological diagnosis which was seen in almost two thirds of the cases i.e. 68.6%, followed by large cell neuroendocrine carcinioma and typical carcinoids seen in 20% and 11.45% of the patients respectively. Liver was seen as the most common metastatic site in 45.8% of cases.[54]

A study done in 2019 by Lijie Song et al retrospectively analyzed clinicopathologic characteristics of 547 patients all pathologically

diagnosed as NETs. Age, sex, primary and metastatic sites, symptoms, pathology, treatment and prognosis were taken into considerations. Wide age range ie 9-87 years was seen. 413 cases were seen in the digestive system with the pancreas, rectum and stomach as the most common primary sites, 74 cases in the lung, 15 in the mediastinum, 8 in unknown sites, and 37 in other sites. Liver, bone and lung were the most frequent sites of metastasis with lymph node metastasis in 82 patients. The most common tumor grade came out to be G1, followed by G2 and G3 in digestive system. In pulmonary NETs, 39 patients had typical carcinoid tumors and 35 had atypical carcinoid tumors. [55]

MATERIAL AND METHODS

The present study was a prospective and retrospective study on histopathological spectrum of neuroendocrine tumors of specimens received in the Department of Pathology, Dayanand Medical College & Hospital, Ludhiana over a period of 5 years [3.5 years retrospectively from 1st July 2015 to 31st December 2018 and 1.5 years prospectively from 1st January 2019 to 30th June 2020]. During this period all the specimens were analysed with respect to light microscopic and IHC findings for neuroendocrine neoplasms.

A total of 52,430 specimens were received during the study period, out of which 123 cases of neuroendocrine neoplasms were diagnosed with respect to light microscopic and immunohistochemical findings.

METHOD OF COLLECTION OF DATA

Inclusion criteria: All the biopsies and resected specimens of IHC proven neuroendocrine tumors.

Exclusion criteria: All tumors which do not show neuroendocrine differentiation by IHC and neuroendocrine hyperplasia .

Duration of study: Retrospective 3 ½ years and prospective 1 ½ years.

Study Design: This is prospective and retrospective study.

Methodology: Biopsies and resected specimens were received in containers containing 10% formalin. Random representative sections were taken from the specimen and then were processed. Paraffin embedding and subsequent staining by Hematoxylin and Eosin was done. IHC markers such as Synaptophysin, Chromogranin A, Neuron specific enolase, CD56 were used. Site specific markers such as TTF-1, CDX2, SATB2, PSA were also used wherever required.

Paraffin Block Tissue Processing

The tissue fixed in 10 % formalin was processed through a series of solutions in the automatic tissue processor as follows:

- Acid formalin for 4 hours
- Formal alcohol for 1 hour

- 80% ethyl alcohol for 1 hour
- 90% ethyl alcohol for 1 hour
- Absolute ethyl alcohol for 1 hour
- Acetone [1st change] for 1 hour
- Acetone [2nd change] for 1 hour
- Xylene [1st change] for 1 hour
- Xylene [2nd change] for 1 hour
- Paraffin [1st change at 58-69°C] for 1 hour
- Paraffin [2nd change at 58-69°C] for 1 hour

The paraffin embedded tissue blocks were cut at 2-3um thickness by rotary microtome. The sections were then transferred onto albunimized slides. These sections were labelled properly with the help of glass marking pencil.

Procedure of Hematoxylin and Eosin staining:

- Slides were kept on hot plate in order to dewax the sections at temperature upto 60ºC.
- Then the slides were deparaffinised in xylene
- Then hydration was done by passing through descending rates of alcohol to water
- The slides were then stained in Harris Hematoxylin for 15 minutes. Sections were washed well in running tap water until sections turned blue. These sections were then differentiated in 1 % acid alcohol [1% HCL in 70% alcohol] for 5-10 seconds, washed well in tap water till sections were again blue for 5 minutes or less.
- These were again stained in 1% Eosin for 10 minutes followed by washing in running tap water for 1-5 minutes.
- Sections were dehydrated once again through ascending grades of alcohol, cleaned in Xylene and mounted with DPX [Distyrenedibutylphtalate xylene] avoiding air bubbles.
- Stained slides were then subjected to microscopic examinations under both [10X] and high power [40X]

Procedure for Immunohistochemical staining:

Immunohistochemistry was done in 123 cases. Suitable blocks were chosen for IHC. The immunohistochemical stains used were Synapotophysin, Chromogranin A, NSE, CD56, TTF-1, CDX2, CK, ER, PR, PAX 8, S-100 etc.

Ventana Benchmark GX was used for immunohistohistochemistry.

Methodology:

IMMUNOHISTOCHEMISTRY-

1. 2-3 um sections were cut and mounted on Poly-L-lysine coated slides. Slides were dried overnight at 37 degree C and dewaxed in xylene and hydrated.
2. Antigen retrieval procedure- The black box was filled 3/4th with citrate buffer and the slide carrier with slides was placed in it. The black box was placed in microwave for 9 mins.
3. Boiling of the solution was observed and the microwave was reset for 6 min and started.
4. The box was removed and cooled under tap water
5. Blocking endogenous peroxidase - Sections were incubated at room temperature in 0.3% H2O2 in methanol for 30-45 min and rinsed in running tap water for 5-10 mins and then dipped in distilled water for 1 min.
6. The slides were placed in Tris buffer [only one change for 5 min].
7. Blocking reagent was applied for 5-10 min and excess of it was wipped off.
8. Primary antibodies were applied covering the whole section and incubated at room temperature for 1 hour.
9. Slides were then washed in Triss buffer for 5 minutes.
10. Ultra Vision One HRP polymer covering the whole tissue was applied and incubated at room temperature for 45 mins.
11. Washing was done in TBS buffer [Tris buffer saline] for 5 min [4 changes].

12. One drop of substrate DAB [3, 3- Diaminobenzidine] solution was applied on each slide covering the whole material and incubated for 10-15 mins, then washed in deiodinized water.
13. Hematoxylin was applied for few seconds and washed under running water.
14. Dry and dehydrate in alcohol and incubate for 2 mins.
15. Mounted in DPX [distyrenedibutyl phthalate xylene]

OBSERVATION AND RESULTS

The present 5 year study [3.5 years retrospective from 1st July 2015 to 31st December 2018 and 1.5 years prospective from 1st January 2019 to 30th June 2020] on histopathological spectrum of neuroendocrine tumors of specimens was conducted in the Department of Pathology, Dayanand Medical College & Hospital, Ludhiana.

During the study period a total of 123 cases of immunohistochemically confirmed neuroendocrine neoplasms were analysed with respect to light microscopic and immunohistochemical findings.

The immumnohistochemical panel for neuroendocrine tumors comprised of antibodies for Synaptophysin, Chromogranin A, CD56 and Neuron Specific Enolase. Other site specific markers such as TTF-1, CEA, and CDX2 were used to know the primary site in cases of metastasis wherever necessary. Markers like CK, CK7, CK20, SATB2, PAX8, S-100, ER, PR, HER2 and PSA were done depending on clinical setting. Clinical and radiological correlation was also done in all the cases to reach at a definite site specific diagnosis.

The Neuroendocrine Neoplasms [NENs] were classified according to 2010 & 2019 WHO Classification of Digestive System Tumors, 2017 WHO Classification of Tumors of Endocrine Organs, 2015 WHO Classification of Tumors of Lung, Pleura, Thymus and Heart, 2012 & 2019 WHO Classification of Breast Tumors, 2016 WHO Classification of Tumors of Urinary System and Male Genital Organs. A total of 52,430 specimens were received during the study period. Out of which 14,180 were malignant specimens with 123 cases diagnosed as neuroendocrine neoplasms which constituted 0.86 % of the malignant specimens.

Age wise distribution of patients with NENs

In the present study, the age of presentation ranged from 11 years to 90 years.

It was observed that the maximum patients were seen between 51-60 years of life with a mean age of 56 years.

Table 1: Age wise distribution of patients with NENs [n=123]

Age Range [years]	No of patients [n=123]
11-20	02 [1.63%]
21-30	05 [4.06%]
31-40	05 [4.06%]
41-50	21 [17.07%]
51-60	43 [34.96%]
61-70	32 [26.02%]
71-80	13 [10.57%]
81-90	02 [1.63%]

Sex distribution of patients of NENs

It was observed that males were affected [73 cases, 59.35%] more commonly than the females [50 cases, 40.65%] with a male to female ratio of 1.4:1.

Table 2: Sex distribution of patients of NENs [n=123]

Sex	No of patients [n=123]	Percentage
Female	50	40.65%
Male	73	59.35%

Immunohistochemistry findings in NENs

In 123 cases, Ki-67 [MIB-1] and CK along with one or more of neuroendocrine markers were done such as Synaptophysin [n=113/123], Chromogranin A [n=120/123], CD56 [n=15/123] and NSE [n=32/123].

Synaptophysin positivity was seen in 101/113 cases [89.38%]. Of the 101 cases dual positivity with Chromogranin A was seen in 83 cases, 4 cases showed additional positivity for CD56, NSE and 4 cases showed dual positivity with NSE whereas, 10 cases were positive for Synaptophysin only.

Chromogranin A was positive in 101/120 cases [84.17%]. Of the 101 cases, 83 cases showed dual positivity with Synaptophysin, four cases showed dual positivity for Chromogranin A and NSE while 14 cases were positive for Chromogranin A only.

Of the 123 cases, two cases with typical histomorphology of NENs showed positivity for NSE and 1 case showed positivity for CD56 with all other markers being negative.

Table 3: Immunohistochemistry findings in NENs [n=123]

IHC marker	Positive	Negative
Synaptophysin [n=113]	101 [89.38%]	12 [10.62%]
Chromogranin A [n=120]	101 [84.17%]	19 [15.83%]
NSE [n=32]	25 [78.12%]	7 [21.88%]
CD56 [n=15]	10 [66.67%]	5 [33.33%]

These cases were distributed according to clinical, radiological findings into neoplasms of primary and metastatic site. Appropriate IHC to locate the primary site was used wherever required.

DISTRIBUTION OF CASES ACCORDING TO PRIMARY AND METASTATIC SITE

Of the 123 cases, 64 [52.03%] cases were diagnosed as primary neuroendocrine neoplasms [NENs] while 59 [47.97%] cases presented as metastatic deposits to various sites.

Of the 123 cases, majority of cases were seen in liver [n=33], gastrointestinal tract [n=31], lymph nodes [n=27] and lung [n=13].

Of the 64 cases of primary neuroendocrine neoplasms, gastrointestinal tract was the commonest primary site [n=30] followed by lung [n=13], pancreas [n=8], adrenal [n=4], breast [n=4], liver [n=2] and one case each of prostrate, gall bladder and retroperitoneal mass.

Of the 59 cases of metatstaic lesions majority were seen in liver [n = 31] followed by lymph nodes [n=27] and gastrointestinal tract [n=1].

These metastatic lesions were analysed using extended IHC panels, correlation with clinical findings and radiological features to reach a definite site specific diagnosis.

Table 4: Distribution of NENs cases according to primary and metastatic site

Location	Primary cases [n=64]	Metastatic cases [n=59]	Total no of cases [n=123]
Liver	02	31	33
GI	30	01	31
Lymph node	00	27	27
Lung	13	00	13
Pancreas	8	00	8
Adrenal	04	00	04
Breast	04	00	04
Prostrate	01	00	01
Gall bladder	01	00	01
Retroperitoneal mass	01	00	01

1. METASTATIC NENs

All the 59 cases were subjected to extended IHC panels including, site specific IHC markers like CDX2/SATB2 [for GI tract], TTF-1 [for lung/ prostate] along with additional markers CK7, CK20, PSA, S-100, PAX 8 etc. to know the primary site. Detailed clinical and radiological correlation was done in order to ascertain the primary site, where the IHC markers were non corroborative.

Out of the 59 cases of metatstaic lesions majority were seen in liver [n=31, 52.54%] followed by lymph nodes [n=27, 45.76 %] and gastrointestinal tract [n=1, 1.70%]. Of the 31 cases with liver lesions, majority [n=14] cases were diagnosed as primary gastrointestinal tract origin, followed by

lung [n=11], pancreas [n=4] and 1 case each from prostrate and thyroid. Of the 27 cases of lymph nodes lesions majority were from lung [n=13], gastrointestinal tract [n=12] and 1 each from thyroid & pancreas. Of the single case from gastrointestinal tract, lung was the primary site.

Thus, out of 59 [47.97%] cases of metastatic NENs deposits, most common primary site was gastrointestinal tract [26] as they showed CDX2/SATB2 [Nuclear] positivity.

Table 5: Distribution of primary site of metastatic deposits of NENs of liver, lymph node and gastrointestinal tract

Site of Metastasis	Primary site
Liver [n= 31]	Gastrointestinal tract -14 Lung -11 Pancreas -4 Prostrate -1 Thyroid -1
Lymph nodes [n= 27]	Lung -13 Gastrointestinal tract -12 Thyroid -1 Pancreas -1
Gastrointestinal Tract [n=1]	Lung -1

Metastatic deposits of NENs of primary lung [25 cases], 17 cases showed TTF-1 [Nuclear] positivity. Rest 8 cases were clinically and radiologically correlated and primary site came out to be lung.

Five cases were diagnosed as metastatic lesions with pancreas as primary as they were CK7 [Cytoplasmic] positive with CK20 [Cytoplasmic] negative along with clinico-radiological correlation.

Two cases showed PAX8 positivity in addition to specific neuroendocrine markers thus confirming the diagnosis as thyroid neuroendocrine tumors metastatic to lymph node and liver respectively. Both cases also had radiologically proven lesion in the thyroid.

One case with past history of carcinoma prostate showed positivity for TTF-1 in addition to specific neuroendocrine markers and was thus diagnosed as primary neurondocrine carcinoma of prostate metastatic to liver despite the lesion being negative for PSA.

Thus, overall of the 123 cases, the maximum number of NENs cases [primary as well as metastatic deposits to other sites] were of gastrointestinal tract [56, 45.53%] followed by lung [38, 30.89%], hepatopancreatobiliary [16, 13.01%], breast [4, 3.25%], adrenal [4, 3.25 %], thyroid [2, 1.63%], prostrate [2, 1.63%] and retroperitoneum [1, 0.81%].

2. PRIMARY NENs

NENs OF GI TRACT

Site

Amongst the total cases [56/123, 45.53%] in gastrointestinal tract maximum number of cases of NENs were seen in small intestine [29, 51.78%] followed by large intestine [16, 28.57%], stomach [6, 10.71%], ampulla [3, 5.36%], appendix [1, 1.79%] and esophagus [1, 1.79%].

Table 6: Distribution of NENs of GI tract according to site [n=56]

Location	Primary [n=30]	Metastasis to liver [n=14]	Metastasis to lymph node [n=12]
Esophagus [n=1, 1.79%]	1	0	0
Stomach [n=6, 10.71%]	5	1	0
Small intestine [n=29, 51.78%]	12	8	9
Large intestine [n=16, 28.57%]	8	5	3
Ampulla [n=3, 5.36%]	3	0	0
Appendix [n=1, 1.79%]	1	0	0

Age Group

Maximum numbers of patients were seen in age group of 51-60 years. Mean age seen was 54.7 years.

Table 7: Age wise distribution of NENs of GI tract [n=56]

Age Range [years]	No of patients [n=56]
21-30	02 [3.57%]
31-40	04 [7.15%]
41-50	15 [26.79%]
51-60	16 [28.57%]
61-70	13 [23.21%]
71-80	06 [10.71%]

Sex

Male predominance was seen in NENs of GI tract with a male to female ratio of 1.9:1.

Table 8: Sex distribution of NENs of GI tract [n=56]

Sex	No of patients [n=56]	Percentage
Female	19	33.93%
Male	37	66.07%

Classification and grading of NENs of the GI tract according to 2010 WHO Classification of Digestive Tumors

Table 9: Classification and grading of NENs of the GI tract according to 2010 WHO Classification of Digestive Tumors

2010 WHO Classification		Cases [n=56]
Well Differentiated	NET Grade 1	30 [53.57%]
	NET Grade 2	4 [7.14%]
Poorly Differentiated	SCNEC	4 [7.14%]
	LCNEC	17 [30.36%]
	MANEC	1 [1.79%]

[NET–Neuroendocrine Tumor, SCNEC-Small cell neuroendocrine carcinoma, LCNEC-Large cell neuroendocrine carcinoma, MANEC- Mixed adenoneuroendocrine carcinoma, MiNEN- Mixed neuroendocrine- non -neuroendocrine neoplasm]

Histological grading was also done according to WHO 2019 classification of digestive tumors to compare the histopathological pattern of NENs of GI tract. In our study the neuroendocrine tumor grade 1, grade 2, grade 3 were found in 30 [53.57%], 4 [7.14%] and 6 [10.72%] cases respectively. Four [7.14%] cases of small cell neuroendocrine carcinoma and 11 [19.64%] cases of large cell neuroendocrine carcinoma were seen. One [1.79%] case of mixed neuroendocrine –non neuroendocrine neoplasms was seen. The most common histological grade was NET, G1 [53.57%] in our study.

Table 10: Classification and grading of NENs of the GI tract according to 2019 WHO Classification of Digestive Tumors

<table>
<tr><th>Terminology</th><th>Differentiation</th><th>Grade</th><th>Cases [n=56]</th></tr>
<tr><td>NET,G1</td><td rowspan="3">Well differentiated</td><td>Low</td><td>30 [53.57%]</td></tr>
<tr><td>NET,G2</td><td>Intermediate</td><td>4 [7.14%]</td></tr>
<tr><td>NET,G3</td><td>High</td><td>6 [10.72%]</td></tr>
<tr><td>NEC, small cell type [SCNEC]</td><td rowspan="2">Poorly differentiated</td><td rowspan="2">High</td><td>4 [7.14%]</td></tr>
<tr><td>NEC, large cell type [LCNEC]</td><td>11 [19.64%]</td></tr>
<tr><td>MINEN</td><td>Well or poorly differentiated</td><td>Variable</td><td>1 [1.79%]</td></tr>
</table>

Comparison between 2010 and 2019 WHO classification of NENs of GI tract

17 cases were diagnosed as large cell carcinoma according to WHO 2010 classification but according to 2019 classification 6 cases were reclassified as NET, G3 on the basis of histomorphological features such as architecture, prominent nucleoli and necrosis. It was observed that the NET, G3 tumors had vesicular nuclei with inconspicuous nucleoli and moderate to abundant amount of cytoplasm with presence of cells in organoid, nests, cords or trabecular pattern instead of diffuse sheets which were seen in neuroendocrine carcinomas.

Large cell neuroendocrine carcinoma cases had vesicular nuclei with prominent nucleoli and abundant amount of cytoplasm. Necrosis was absent in almost all cases of neuroendocrine tumor grade 3. Ki-67 index was also taken into consideration for reclassifying these cases. Ki-67 index in NET, G3 cases was seen around 22 to 50% whereas neuroendocrine carcinomas had a Ki-67 index between 50-100%.

Site wise grading of NENs of GI tract

Maximum number of NENs of GI tract were from small intestine [29] followed by large intestine [16] and least number of cases from ampulla [3] and appendix [1].

Most common histological grade seen in small intestine was NET, G1 [48.28%] followed by large cell neuroendocrine carcinoma [20.69%].

Table 11: Site wise grading of NENs of GI tract

LOCATION	NET, G1 [n=30]	NET, G2 [n=4]	NET,G3 [n=6]	SCNEC [n=4]	LCNEC [n=11]	MiNEN [n=1]
Esophagus [n=1]	0	0	0	1	0	0
Stomach [n=6]	4	1	0	0	1	0
Small Intestine [n=29]	14	2	4	2	6	1
Large Intestine [n=16]	9	1	2	1	3	0
Ampulla [n=3]	3	0	0	0	0	0
Appendix [n=1]	0	0	0	0	1	0

Morphology

The most common architectural pattern seen in well differentiated neuroendocrine neoplasms [NET G1, G2, G3] was nests and zell ballen. Other architectural forms which were seen were glandular, trabeculae, cords, islands, cribrifrom, gyriform and acinar. Mixed patterns were also observed. Occasional sheeting pattern was also seen in these neoplasms. Well differentiated neuroendocrine neoplasms had round nuclei, plasmacytoid morphology, stippled chromatin or vesicular nuclei with inconspicuous nucleoli and moderate to abundant amount of cytoplasm.

Whereas, the poorly differentiated neoplasms [NEC] had sheets followed by nests as the most common architectural pattern. Cell size varied from medium to large which was greater than 3 resting lymphocytes.

Large cell neuroendocrine carcinoma cases [11] had large cells with vesicular nuclei, prominent nucleoli and abundant amount of cytoplasm. Necrosis was seen in large cell neuroendocrine carcinomas, with 5 cases showing focal areas of necrosis and 6 showing extensive necrosis.

Cases of small cell neuroendocrine carcinoma [4] had hyperchromatic small, round nuclei with spindling and scant cytoplasm. Mixed neuroendocrine with non-neuroendocrine component was seen in 1 case with adenocarcinoma as the non-neuroendocrine component.

Immunohistochemical expression of NENs of GI tract

NET, G1 - Dual positivity for Synaptophysin and Chromogranin A was seen in 24 out of 30 cases of NET, G1. In 3 cases Synaptophysin was not done but it showed positivity for Chromogranin A along with NSE in one of the case. Two cases were positive for Synaptophysin and negative for Chromogranin A. One case showed positivity for Chromogranin A only.

NET, G2 - 4 cases were seen, out of which dual positivity for Chromogranin A and Synaptophysin was seen in 2 cases with one case each positive for Synaptophysin and Chromogranin A respectively.

NET, G3 - 6 cases were seen in this study out of which 3 cases showed dual positivity for Chromogranin A and Synaptophysin. Two cases were positive for Synaptophysin and NSE and negative for Chromogranin A. One case was positive for Chromogranin A only.

Small cell neuroendocrine carcinoma - Dual positivity for Synaptophysin and Chromogranin A in 2 cases was seen out of 4 cases. One case showed positivity for Synaptophysin only. The 4th case showed positivity for NSE and CD56 with all other markers as negative.

Large cell neuroendocrine carcinoma - 11 cases were seen with 5 having dual positivity of Synaptophysin and Chromogranin A. One case was positive for Synaptophysin, NSE & CD56 with Chromogranin A negativity and one case was positive for Synaptophysin only with all other markers being negative. Two cases showed positivity for Chromogranin A

and were negative for Synaptophysin. One case showed positivity for NSE and was negative for both Synaptophysin and Chromogranin A. One case was positive for Chromogranin A and NSE but showed negativity for Synaptophysin.

Mixed neuroendocrine- non- neuroendocrine neoplasm – One case was seen which was positive for both Synaptophysin and Chromogranin A.

Thus, the cases were classified as NET and NEC on the basis of morphology which resembled a neuroendocrine neoplasm and positive immunohistochemical staining with a neuroendocrine marker such as Synaptophysin / Chromogranin A / NSE.

Table 12: Immunohistochemical expression of NENs of GI tract

IHC marker	Positive cases					
	NET G1	NET G2	NET G3	SCNEC	LCNEC	MiNEN
Synaptophysin [n=52]	26/27	3/3	5/6	3/4	7/11	1/1
Chromogranin A [n=56]	28/30	3/4	4/6	2/4	8/11	1/1
NSE [n=16]	6/7	0/0	2/4	1/1	3/4	0/0
CD56 [n=4]	2/2	0/0	0/0	1/1	1/1	0/0

NENs OF LUNG

Out of total 123 cases, 38 were seen as NENs of lung including primary and metastasis.

Table 13: NENs of lung [n=38]

	Primary	Metastasis to liver	Metastasis to lymph node	Metastasis to GI tract
Lung [n=38]	13	11	13	1

Age group

Maximum cases of neuroendocrine neoplasms of lung were seen in the age range of 51-60 years with a mean age of 58.9 years.

Table 14: Age wise distribution of NENs of lung [n=38]

Age Range [years]	No. of patients [n=38]
11-20	01 [2.63%]
21-30	00 [0%]
31-40	01 [2.63%]
41-50	03 [7.89%]
51-60	19 [50%]
61-70	08 [21.06%]
71-80	04 [10.53%]
81-90	02 [5.26%]

Sex

A male predominance [25, 65.79%] was seen in NENs of lung with a male to female ratio of 1.9:1.

Table 15: Sex distribution of NENs of lung [n=38]

Sex	No of patients [n=38]	Percentage
Female	13	34.21%
Male	25	65.79%

Histological grading of NENs of lung according to 2015 WHO for Lung, Pleura, Thymus and Heart.

Maximum, number of NENs of lung were seen of small cell carcinoma [15 cases, 39.47%], followed by large cell neuroendocrine carcinoma [13 cases, 34.21%], atypical carcinoid [6 cases, 15.79%] and typical carcinoid [4 cases, 10.53%].

Table 16: Histological grading of NENs of lung according to 2015 WHO for Lung, Pleura, Thymus and Heart

WHO Classification	No of cases [n=38]
Typical Carcinoid	4 [10.53%]
Atypical carcinoid	6 [15.79%]
Small cell carcinoma	15 [39.47%]
Large cell neuroendocrine carcinoma	13 [34.21%]

Morphology

Architectural pattern seen in typical carcinoid and atypical carcinoids were nests, cords and islands. They had round nuclei, stippled chromatin or vesicular nuclei with inconspicuous nucleoli and moderate to abundant amount of cytoplasm. Necrosis was absent in the cases of typical carcinoid [4] but was seen in cases of atypical carcinoid [6]. Mitosis was <2 mitosis /2mm^2 in cases of carcinoid and 2-10 mitosis /2mm^2 in atypical typical carcinoid cases.

NEC had sheets followed by nests as the most common architectural pattern. Cell size varied from medium to large which was greater than 3 resting lymphocytes.

Large cell neuroendocrine carcinoma cases [13] had cells which were larger than diameter of 3 resting lymphocytes, with vesicular nuclei, prominent nucleoli and abundant amount of cytoplasm. Necrosis was also seen.

Cases of small cell carcinoma [15] had hyperchromatic small, round nuclei with spindling and scant cytoplasm.

Immunohistochemical marker expression in NENs of lung:

Typical carcinoid - All 4 cases showed dual positivity for Chromogranin A and Synaptophysin with 1 case being positive for NSE also. TTF-1 was negative in all the cases. However, on clinical and radiological correlation the primary was found to be in lung.

Atypical carcinoid - Out of 6 cases, 4 cases showed Synaptophysin and Chromogranin A dual positivity with 1 case showing positivity for Chromogranin A and 1 case for Synaptophysin only. 4/6 cases showed positivity for TTF-1. The remaining 2 cases with TTF-1 negativity were clinically and radiologically correlated and lesion was found to be in lung.

Small cell carcinoma - 15 cases were seen out of which 12 cases showed dual positivity of Chromogranin A and Synaptophysin. One case was positive for Chromogranin A and NSE, 1 case showed positivity for

Synaptophysin only and CD56 positivity was seen in 1 case. TTF-1 was positive in 11 /15 cases of small cell carcinoma of lung. The clinicoradiological correlation was made in remaining four TTF-1 negative cases and the primary lesion came out to be lung.

Large cell neuroendocrine carcinoma - Out of 13 cases, Synaptophysin and Chromogranin A was dual positive in 9 cases with 2 cases each being positive for Chromogranin A and Synaptophysin only. TTF-1 was seen positive in 5/13 cases with the rest 8 cases being radiologically and clinically correlated and primary lesion was found in lung.

Thus, in our study positivity for Synaptophysin and Chromogranin A was seen in almost all the cases of NENs of lung. TTF-1 expression was negative in all the cases of typical carcinoid. 66 % cases of atypical carcinoid, 73.3% cases of small cell carcinoma and 38 % cases of large cell carcinoma were positive for TTF-1.

Table 17: Immunohistochemical marker expression in NENs of lung

IHC marker	Positive cases			
	Typical Carcinoid	Atypical carcinoid	Small cell carcinoma	Large cell neuroendocrine carcinoma
Synaptophysin [n=35]	4/4	5/5	13/14	11/12
Chromogranin A [n=35]	4/4	5/5	13/14	11/12
NSE [n=6]	1/1	0/0	2/3	2/2
CD56 [n=4]	0/0	0/0	2/4	0/0
TTF-1 [n=38]	0/4	4/6	11/15	5/13

NENs of Hepatopancreatobiliary organs

In present study there were 11 cases of primary NENs of hepatopancreatobiliary organs and 5 cases of metastatic deposits. Out of 5 cases of metastatic deposits, 4 were of pancreatic neuroendocrine neoplasm metastasizing to liver and one was metastasis to lymph node.

Table 18: NENs of hepatopancreatobiliary organs [primary and metastasis, n=16]

Location	Primary	Metastasis to liver	Metastasis to Lymph node
Pancreas	8	4	1
Liver	2	-	-
Gall bladder	1	-	-

Age group

Maximum cases were seen between range of 61-70 years with mean age of 56.7 years.

Table 19: Age wise distribution of NENs of hepatopancreatobiliary organs [n=16]

Age Range [years]	No. of Patients [n=16]
11-20	01 [6.25%]
21-30	01 [6.25%]
31-40	00
41-50	02 [12.5%]
51-60	03 [18.75%]
61-70	07 [43.75%]
71-80	02 [12.5%]
81-90	00

Sex

A female predominance [10, 62.5%] was seen with a female to male ratio of 1.6:1.

Table 20: Sex distribution of NENs of hepatopancreatobiliary organs [n=16]

Sex	No of patients [n=16]	Percentage
Female	10	62.5
Male	6	37.5

Histological grading of NENs of liver according to 2010 WHO of Digestive System Tumors:

Out of the 2 primary cases of neuroendocrine neoplasms of liver 1 was of neuroendocrine tumor grade 1 and second was of large cell neuroendocrine carcinoma. These cases were also graded according to WHO 2019 classification of neuroendocrine neoplasm of hepatopancreatobiliary organs and were classified as neuroendocrine tumor grade 1 and large cell neuroendocrine carcinoma.

Table 21: Histological grading of NENs of liver according to 2010 WHO of Digestive System Tumors

2010 WHO Classification		Cases [n-2]
Well Differentiated	NET Grade 1	1 [50%]
	NET Grade 2	0
Poorly Differentiated	SCNEC LCNEC	0 1 [50%]
	MANEC	0

Histological grading of NENs of pancreas according to 2017 WHO of Endocrine Organs

The maximum number [5/13] of cases of NENs seen in pancreas were of neuroendocrine grade 1 followed by 3 cases each of neuroendocrine grade 2 and grade 3. Two cases were of small cell neuroendocrine carcinoma.

Similar classification is followed for pancreatic neuroendocrine neoplasm in 2019 WHO Classification of Digestive Tumors.

One case of large cell neuroendocrine carcinoma was seen in gall bladder.

Table 22: Histological grading of NENs of pancreas according to 2017 WHO of Endocrine Organs

<table>
<tr><th>Terminology</th><th>Differentiation</th><th>Grade</th><th>Cases [n=13]</th></tr>
<tr><td>NET,G1</td><td rowspan="3">Well differentiated</td><td>Low</td><td>5 [38.46%]</td></tr>
<tr><td>NET,G2</td><td>Intermediate</td><td>3 [23.08%]</td></tr>
<tr><td>NET,G3</td><td>High</td><td>3 [23.08%]</td></tr>
<tr><td>NEC, small cell type [SCNEC]</td><td rowspan="2">Poorly differentiated</td><td rowspan="2">High</td><td>2 [15.38%]</td></tr>
<tr><td>NEC, large cell type [LCNEC]</td><td>0</td></tr>
<tr><td>MiNEN</td><td>Well or poorly differentiated</td><td>Variable</td><td>0</td></tr>
</table>

Out of total of 16 cases of hepatopancreatobiliary organs i.e. 16 according to 2019 WHO classification of neuroendocrine neoplasms of GI tract and hepatopancreatobiliary organs 6 were of neuroendocrine grade 1, three each of neuroendocrine grade 2 & 3, two cases each of small cell neuroendocrine carcinoma and large cell neuroendocrine carcinoma.

Morphology

Well differentiated neuroendocrine neoplasms [NET G1, G2, G3] had nests and zell ballen as most common architectural pattern. Other architectural patterns which were seen were glands, trabeculae, cords, islands, cribrifrom, gyriform and acinar. Well differentiated neuroendocrine neoplasms had round nuclei, stippled chromatin or vesicular nuclei with inconspicuous nucleoli and moderate to abundant amount of cytoplasm.

Large cell neuroendocrine carcinoma cases [2] had large cells with vesicular nuclei, prominent nucleoli and abundant amount of cytoplasm.

Cases of small cell neuroendocrine carcinoma [2] had hyperchromatic small, round nuclei with spindling and scant cytoplasm.

Immunohistochemical expression of NENs of hepatopancreatobiliary organs:

NET, G1- 6 cases were seen having dual positivity of Chromogranin A and Synaptophysin in 4 cases. One case showed positivity for CD56, NSE and Synaptophysin with Chromogranin A negativity. One case was positive for Chromogranin A with Synaptophysin negativity.

NET, G2- Out of 3 cases, positivity for Chromogranin A, Synaptophysin was seen in 2 cases with third being positive for Synaptophysin only.

NET, G3- 3 cases were seen. Out of which 2 cases showed dual positivity for Chromogranin A and Synaptophysin with 3rd being positive for Chromogranin A and NSE.

Small cell neuroendocrine carcinoma - Out of 2 cases, Chromogranin A positivity was seen in one case with the other being positive for NSE.

Large cell neuroendocrine carcinoma – 2 cases were seen, 1 showed dual positivity of Chromogranin A and Synaptophysin. The 2nd case was positive for Chromogranin A only.

Table 23: Immunohistochemical expression of NENs of hepatopancreatobiliary organs

IHC marker	Positive cases				
	NET G1	NET G2	NET G3	SCNEC	LCNEC
Synaptophysin [n=14]	5/5	3/3	2/3	0/0	1/1
Chromogranin A [n=16]	5/6	2/3	3/3	1/2	2/2
NSE [n=6]	2/2	1/1	1/1	1/2	0/0
CD56 [n=3]	1/2	0/0	0/0	0/1	0/0

OTHERS

ADRENAL - Four cases seen in adrenal were diagnosed as Pheochromocytoma. They showed zell ballen pattern of tumor cells and showed positivity for Synaptophysin, Chromogranin A, S-100 [Nuclear] in all the 4 cases.

BREAST - Four cases were seen, with 3 cases being of invasive duct carcinoma with neuroendocrine differentiation and one case of well differentiated neuroendocrine tumor according to WHO 2012 and 2019 classification of Breast Tumors. All 4 cases were positive for Chromogranin A with ER and PR positivity and 3 cases were positive for Synaptophysin.

THYROID -Two cases of metastasis of medullary carcinoma of thyroid were seen. One seen as metastasis to lymph node and showed positivity for Chromogranin A, Synaptophysin and CK. Second case was seen in liver with Synaptophysin, CD56, NSE, PAX8 positivity. Calcitonin was also positive in both cases.

PROSTRATE - Out of 2 cases of prostrate 1 was seen as primary site and 2nd was metastasis to liver of small cell neuroendocrine carcinoma with PSA negativity and Synaptophysin with NSE positivity. The patient was a known case of carcinoma prostrate. Primary case was of mixed acinar adenocarcinoma and small cell neuroendocrine carcinoma with Synaptophysin, Chromogranin A, PSA and GATA 3 positivity.

RETROPERITONEUM - A solitary case of paraganglinoma was diagnosed with Synaptophysin and NSE positivity. S-100 positivity was also seen with CK being negative.

DISCUSSION

Neuroendocrine cells seen predominantly in the gastrointestinal tract, in pancreas as islets of Langerhans and in the bronchopulmonary system leads to development of neuroendocrine tumors.[44] The incidence of neuroendocrine neoplasms is increasing due to increased awareness among the physicians and improved diagnostic techniques.

The present study was a prospective and retrospective study on histopathological spectrum of neuroendocrine tumors of specimens received in the Department of Pathology, Dayanand Medical college & Hospital, Ludhiana over a period of 5 years [3.5 years retrospectively from 1st July 2015 to 31st December 2018 and 1.5 years prospectively from 1st January 2019 to 30th June 2020]. During this period 123 cases of neuroendocrine neoplasms were analysed with respect to light microscopic and immunohistochemical findings.

A total of 52,430 specimens were received during the study period, out of which 14,180 were malignant cases and 123 cases were diagnosed as neuroendocrine neoplasms which constituted 0.86% of the malignant neoplasms. This finding is similar to the incidence of NENs as a whole entity i.e. 0.86% of total malignancies as reported by National Cancer Institute of Egypt.[54] Similarly, neuroendocrine neoplasms accounted for 0.5% of all malignancies according to a study done by B.G. Taal et al [56].

A wide range of age of presentation of patients with neuroendocrine neoplasms was seen ranging from 11 years to 90 years with a mean age of 56 years. Maximum number of patients were having average age between 51 to 60 years in our study. Similar findings were noted by Lijie Song et al [55], Mauricio P. Pinto et al [57] with mean age of 50.2 ± 13.8 and 53 years respectively in their studies.

In our study males had higher incidence of neuroendocrine neoplasms ie 59.35 % with male to female ratio of 1.4:1. It is in accordance with study done by Josep Darba et al [58] which showed 57.7% of males having neuroendocrine neoplasms. Rahul Suhas Kulkarni et al [59] also did a

study in which male preponderance [M: F = 1.85:1] of neuroendocrine neoplasms was seen. M:F ratio of 1.5: 1 was seen by Umbreen Hafeez et al [60] which is in accordance to our study.

Immunohistochemistry

Immunohistochemistry for neuroendocrine markers is required to confirm the neuroendocrine nature of the tumor. Synaptophysin and Chromogranin A are the traditional neuroendocrine markers used.[12]

Felipe Silveira et al [51] in a study on neuroendocrine neoplasms had positivity of Synaptophysin in 93.9% and Chromogranin A in 88.8% of the cases. S Felder et al [61] in a study on gastric neuroendocrine neoplasms found that 98% and 93.2% of these tumors were positive for Synaptophysin and Chromogranin A respectively.

Similar findings were seen in present study in which Synaptophysin was positive in 89.3% of neuroendocrine neoplasms and Chromogranin A positivity was seen in 84.17%. Thus, positive immunohistochemical staining for Chromogranin A, Synaptophysin provides necessary evidence for the definitive diagnosis of a tumor of neuroendocrine origin.

Metastatic Neuroendocrine Neoplasms

Neuroendocrine neoplasms are a heterogeneous group of tumors with potentially malignant behavior. Fifty nine [47.97%] cases were seen as metastatic deposits of neuroendocrine neoplasms in our study, which is similar to a study done by Umbreen Hafeez et al [60] in which 53.1% patients were having metastatic disease at the time of presentation. Rahul Suhas Kulkarni et al[59] had 66 % of cases presenting as metastasis. Such high number of cases of metastatic disease at presentation can be due to delayed presentation to the health-care facilities and nonspecific nature of symptoms.

The most common site of metastasis in neuroendocrine neoplasms is liver according to various studies, all the diagnostic methods should be used to find their primary origin, which is important for therapeutic decisions. The most common site of metastasis was liver [52.54%] followed by lymph

node [45.76%] in the present study which is similar to a study done by Felipe Silveria et al [51] in which maximum cases of metastasis were seen in liver [50%]. Similarly, Meng Zhang [50] et al in a study of gastropancreatic neuroendocrine neoplasms found liver [61.5%] to be the commonest site of metastasis.

Primary Neuroendocrine neoplasms

Neuroendocrine neoplasms can occur anywhere in the body but the most common site is gastrointestinal tract, pancreas and the lungs. Gastroenteropancreatic neuroendocrine neoplasms [GEP-NENs] play a central role as they constitute about 70% of all NENs.

Gastrointestinal tract [45.53%] had the maximum number of cases of neuroendocrine neoplasms [NENs] followed by lung [30.89%] in our study which is in accordance with study of Meng zhang et al [50], in which maximum cases were located in the digestive tract ie stomach [24.3%] followed by rectum [24.1%]. Similarly Umbreen Hafeez et al [60] found small intestine [32.9%] to be the most common site closely followed by the lung [27.8%].

However, in a study done by Raziye Boyar Cetinkaya et al [62] lung [48.1%] was the commonest primary site followed by gastroenteropancreatic system [18%]. Neuroendocrine tumors can affect any organ, gastrointestinal tract and lung being the most commonly affected organs and the former can alternate its position with the latter i.e. lung. Ethnic, regional and sample-size differences may lead to differences in the reported distribution of the primary sites of neuroendocrine neoplasms. So, it is important to determine the primary site of metastatic deposits as it can affect the choice of treatment and prognosis.

Gastrointestinal Tract

The incidence of gastrointestinal neuroendocrine neoplasms in particular has been increasing throughout the last decades. This can probably be due to increased awareness and use of diagnostic modalities like

endoscopy and a standardized histopathological and immunohistochemical work-up.

Distribution of 56 cases of gastrointestinal tract in the present study was found to be - small intestine [29, 51.78%], large intestine [16, 28.57%], stomach [6, 10.71%], ampulla [3, 5.36%], appendix [1, 1.79%] and esophagus [1, 1.79%]. Oyvind Hauso et al [63] with the data of Norwegian Registry of Cancer [NRC] and Lombard-Bohasa et al [64] found the most common site for neuroendocrine neoplasms to be small intestine in their respective studies i.e. 26%, 43%. Another study of carcinoid tumors of gastrointestinal tract by Scott N. Pinchot et al [65] also found small intestine [45%] as the most common site, followed by the rectum. While in another study by Amarapurkar DN et al [66] the commonest site was stomach [30.2%] followed by pancreas [23.3%] and duodenum. These differences may be due to ethnic and environmental influences or due to small number of cases in their study as compared to ours.

The mean age at diagnosis of neuroendocrine neoplasms [NENs] of GI tract in present study was 54.7 years with the age ranging between 21 and 80 years. Most of the studies correlated with our study having average age between 51 and 60 years. Rothenstein et al [67] and Melinda A. Maggard et al [68] found the mean age of presentation of gastrointestinal tract neuroendocrine neoplasms to be 56.3 and 60.9 years respectively. We found males to be more commonly affected than females by gastrointestinal tract NENs with a male to female ratio of 1.9:1. Male preponderance was also observed by Amarapurkar DN et al [66] and Niederle etal [69] i.e. M: F = 2.5:1, 1.08:1 respectively in their studies.

Neuroendocrine neoplasms were divided into well-differentiated and poorly differentiated neoplasms based on the Hematoxylin-Eosin [H&E] appearance of the tumor, mitosis, and Ki-67 immunohistochemistry.[12]

The most common histological grade of NENs of GI tract was Neuroendocrine Tumor G1 [53.57%] in present study. Similarly, Martin b. Niederle et al [69] also had majority of neuroendocrine tumors of GI tract as

G1 [59.7%] in their study done on gastropancreatic tumors according to 2010 WHO classification of NENs of GI tract which is equal to NET, G1 cases according WHO 2019 classification of digestive tumors. S Felder et al[61] also found G1 [60.6%] to be the most common grade of neuroendocrine neoplasm.

NET, G1– Positivity rate of Synaptophysin, Chromogranin A in NET, G1 was 96 %, 93% respectively in the present study which is similar to a study done by S Felder et al [61] which showed 98.3% and 100% positivity of Synaptophysin and Chromogranin A in NET, G1 tumors. In another study by Tian Ming XU et al [70], positivity of Synaptophysin and Chromogranin A was 78.6% and 71.1% in G1 tumors. These differences in the observations might be due to less number of cases in the study by Tian Ming XU i.e. 14 cases of G1 compared to 30 cases in our study.

NET, G2 - We had positivity rate of 100 % for Synaptophysin and 75 % for Chromogranin A in NET, G2. Variable results have found in the literature regarding positivity of Synaptophysin and Chromogranin A in NET, G2 tumors. Tian Ming XU et al[70], S Felder et al [61] found 77.3%, 100% positivity for Synaptophysin and 68.2%, 100% positivity for Chromogranin A in NET, G2 in their respective studies. The differences of positivity can be due to very less number of cases of NET, G2 in present study.

NET, G3- Present study had positivity rate of 83% for Synaptophysin and 66% for Chromogranin A in NET, G3 category which is similar to 100% and 90.5 % positivity of Synaptophysin and Chromogranin A seen in study done by S Felder et al.[61] Similar findings were seen in study by Tian Ming XU et al [70] i.e. 90.5%, 61.9 % positivity of Synaptophysin and Chromogranin A.

Small cell neuroendocrine carcinoma- Present study had 75%, 50% positivity for Synaptophysin and Chromogranin A respectively. In a study by Anna Fen –Yau Li et al [71] 100% positivity for Synaptophysin was seen in small cell neuroendocrine carcinoma which is discordant with our study

however, Chromogranin A had 61.9% positivity which is similar to our study.

Large cell neuroendocrine carcinoma - Positivity of 63% for Synaptophysin was seen in cases of large cell neuroendocrine carcinoma and 72% for Chromogranin A in our study which is discordant with the data from WHO Digestive System stating that Synaptophysin positivity is a consistent feature, whereas Chromogranin A expression may not generally be seen.[14] These variation can be due to the large cohort used.

Prognosis of NETs varies widely and it depends on the organ of origin, its hereditary association and proliferative grading of the neoplasms by WHO, whereas NECs uniformly have a poor prognosis. So it is important to differentiate between the two entities.

Lung

Pulmonary neuroendocrine tumors represent a morphologic spectrum of tumors ranging from the well-differentiated typical carcinoid tumor, to atypical carcinoid tumor, to neuroendocrine carcinomas composed of small-cell carcinoma and large-cell neuroendocrine carcinoma.

The mean age of diagnosis of NENs of lung in our study was 58.9 years. Maximum number of patients were seen between age range of 51-60 years which is similar to many other studies.

Magdy Saber et al [54] evaluated 35 patients with NENs of lung, the mean of patients age came out to be 53± 11.2 years which is in concordance with our study. A mean age of 57.9 years was seen in a study done by William A. Cooper et al [72] which is also similar to our study.

A male predominance [65.7%] was seen in our study of NENs of lung which is similar to study done by Magdy Saber et al [54] where males constituted 88.6%.

Maximum, number of NENs of lung in the present study were of small cell carcinoma [15 cases, 39.47%], followed by large cell neuroendocrine carcinoma [13 cases, 34.21%] which is in concordance with many studies. YI-CHEN YEH et al [73] found the most common histology of small cell lung

carcinoma [38.9%] in their study. Similarly, Magdy Saber et al [54] found small cell carcinoma to be the prevalent histology in 68.6% of the cases, followed by large cell neuroendocrine carcinoma in 20% of the cases. Xiaoqi Lin et al[74] in a study on 60 cases of pulmonary neuroendocrine carcinoma, found 30 [50%] cases of small cell carcinoma.

Predominance of small cell lung carcinoma [SCLC] amongst lung neuroendocrine neoplasms in the present study is similar to the worldwide incidence of SCLC being the most common subtype.

Carcinoid - In the present study, all 4 cases of typical carcinoid showed dual positivity for Chromogranin A and Synaptophysin. These findings are similar to a study done by Marzia Bonato et al [75], in which positivity for Chromogranin A and Synaptophysin was seen in all 81 cases of typical carcinoids. Similarly in a study by V. Grøndahl et al[76] found Synaptophysin positivity in 100% cases of typical carcinoid.

All cases of atypical carcinoid in present study showed 100% positivity for Chromogranin A and Synaptophysin which is similar to findings of V. Grøndahl et al[76] which had 100% positivity of Synaptophysin in atypical carcinoids. YI-CHEN YEH et al [73] also had 100% positivity for both these markers in cases of atypical carcinoid.

TTF-1 came out to be positive in 40% cases of lung carcinoid in our study which is similar to study done by Chen Zhang et al [77] which showed positivity of TTF-1 for 35% cases of typical and atypical carcinoid combined. Amitabh Srivastava et al [78] found 35% of pulmonary carcinoids to be TTF-1 positive.

Once the diagnosis of typical versus atypical carcinoid is established, impact on prognosis and therapy is significant. In typical carcinoids metastasis to the lymph node may not have an impact on overall survival and so may not require adjuvant therapy. However, atypical carcinoid may need therapy with completion lobectomy or wedge resections even if without nodal disease.

Small cell carcinoma - In the present study, positivity of Chromogranin A and Synaptophysin was seen in 92% of the cases. Vandana Bhatti et al [79] found 100% positivity for Synaptophysin and Chromogranin A in small cell carcinomas. TTF-1 was positive in 73.3 % of small cell carcinoma lung in our study which is similar to TTF-1 positivity of 81%, 90% in studies done by O Kaufmann & M Dietel et al [80] and Xiaoqi Lin et al [74] respectively.

Large cell neuroendocrine carcinoma - Positivity for Synaptophysin and Chromogranin A in large cell neuroendocrine carcinoma in our study was 91% for both. In a study done by G. Rossi et al [81] positivity of Synaptophysin and Chromogranin A was 55%, 85% respectively. TTF-1 positivity was seen in 83% of large cell neuroendocrine carcinoma which is higher than the positivity of TTF-1 in our study i.e. 38%. It can be due to large number of cases i.e. 47 analyzed in their study as compared to our study i.e. 12. According to data presented by WHO lung TTF-1 positivity can be seen in less than 50% cases of large cell neuroendocrine carcinoma.[7]

The distinction of small-cell carcinoma from large-cell neuroendocrine carcinoma, is of relevance to the therapeutic approach to these tumor types despite both being in the high-grade group.

Hepatopancreatobiliary organs

Maximum cases of NENs were seen between age range of 61-70 years in hepatopancreatobiliary organs with a mean age of 56.7 years in our study. Yue Xu et al [82] found 56 years as the mean age of diagnosis. Matias Riihimäki et al [83] found the mean age of diagnosis to be 68 years.

Female preponderance in hepatopancreatobillary organs of 62.5 % was seen in the present study. Similar findings have been found by other authors as well. Female preponderance [52.5%] was seen by Yue Xue et al [82] in pancreatic neuroendocrine tumors. Satoshi Shiba et al [84] also did a study on pancreatic neuroendocrine neoplasms and found a female preponderance of 52%.

The maximum number of cases of NENs seen in pancreas of present study were of neuroendocrine grade 1 [5, 38.46%] followed by neuroendocrine grade 2 [3, 23.08%] and grade 3 [3, 23.08%] which is similar to a study by Feng JIN et al[85] who reviewed 31 cases of pancreatic neuroendocrine neoplasms and the maximum cases were of NET, G1 i.e. 19.

In the present study 2 cases of primary hepatic neuroendocrine neoplasms were seen, one of NET, G1 and other of large cell neuroendocrine carcinoma. Amir Parray et al[86] studied 13 cases of primary hepatic neuroendocrine neoplasms. In their study neuroendocrine tumors were more common than neuroendocrine carcinomas.

NET, G3 do not respond to platinum-based chemotherapy like NECs so it is important to differentiate the two.

In a study by Yugang Cheng et al [87] 100% positivity of Synaptophysin and 96.2% positivity of Chromogranin A was seen in pancreatic neuroendocrine neoplasms which is similar to our findings in which 100 % positivity of Synaptophysin and 83 % positivity of Chromogranin A was seen amongst hepatopancreatobillary neuroendocrine neoplasms.

OTHERS

ADRENAL - A study by MAGDALENA BIAŁAS et al[88] on 64 cases of pheochromocytoma showed dual positivity by Synaptophysin and Chromogranin A in all the cases with S-100 positivity of sustentacular cells. Similar findings were seen in our study in which positivity for Synaptophysin, Chromogranin A and S-100 was seen in 100% of the cases.

BREAST – There were 4 cases of breast carcinoma with 3 cases being of invasie duct carcinoma with neuroendocrine differentiation and one case of well differentiated neuroendocrine tumor. All 4 cases were positive for Chromogranin A with 3 cases showing Synaptophysin positivity. In a study on primary neuroendocrine neoplasms of breast by Burcin Özdirik et al [89]

all cases were positive for Synaptophysin with Chromogranin A positivity seen in 4 out of 5 cases in that study.

Thus, NENs are increasing in incidence and have a heterogenous nature. Symptoms can be missed, resulting in late diagnosis. Pathologic classification is complex but is the key to understand the tumor behavior. The data suggests that tumors primarily affect the gastrointestinal system, pancreas and lung. By integrating morphology with immunohistochemistry a definitive diagnostic information can be provided.

SUMMARY AND CONCLUSION

Neuroendocrine neoplasms are known to be rare tumors and have shown an increasing incidence over the recent decades, most probably due to increased awareness among the physicians and improved diagnostic techniques.

In this study we analysed the histopathological and immunohistochemical characteristics of 123 neuroendocrine neoplasms and the findings are -

1. A total of 14,180 malignant specimens were received during the study period, out of which 123 cases were diagnosed as neuroendocrine neoplasms constituting 0.86 % of the malignancies.
2. The age of presentation ranged from 11 to 90 years with a mean age of 56 years. Maximum patients were seen between 51 to 60 years.
3. Males were more commonly affected [59.3%] by neuroendocrine neoplasms than females with a male to female ratio of 1.4:1.
4. The basic two immunohistochemical markers i.e. Synaptophysin was positive in 89.3 % cases and Chromogranin A was positive in 84.17 % cases. Other neureoendocrine markers such as NSE and CD56 were used.
5. Fifty nine cases i.e. 47.97 % cases were seen as metastatic deposits of neuroendocrine neoplasms in our study. The most common site of metastasis was liver followed by lymph node in present study.
6. Maximum cases of neuroendocrine neoplasms were seen in gastrointestinal tract i.e. 56 [45.5%] followed by lung [30.8%]. Amongst the 56 cases of gastrointestinal tract maximum cases were seen in small intestine [51.78%] followed by large intestine [28.57%]. The age range of neuroendocrine neoplasms of gastrointestinal tract in present study was 21 to 80 years with average age ranging between 51 to 60 years. Males had a higher incidence of NENs of gastrointestinal tract [66.07%] and a male to female ratio of 1.9:1.
7. Most common histological grade of neuroendocrine neoplasms of gastrointestinal tract was Neuroendocrine Tumor G1 [NET, G1] i.e.

53.57%. The positivity rates for Synaptophysin, Chromogranin A were high in neuroendocrine tumors as compared to neuroendocrine carcinomas.

8. Maximum number of patients of lung neuroendocrine neoplasms were seen between age group of 51-60 years. Male predominance of 65.79 % was seen in lung neuroendocrine neoplasms. Most common neuroendocrine neoplasms was small cell carcinoma i.e. 39.47%.
9. In lung NENs, typical and atypical carcinoids showed 100% positivity with Synaptophysin and Chromogranin A. Positivity of TTF-1 was seen in 40% cases of carcinoid . Small cell carcinoma showed a positivity of 92% for Synaptophysin and Chromogranin A, whereas large cell neuroendocrine carcinoma had a positivity of 91 % for Synaptophysin and Chromogranin A each. TTF-1 was positive in 73.3% of small cell carcinomas and 38% for large cell carcinomas.
10. Maximum number of cases of hepatopancreatobiliary neuroendocrine neoplasms were seen in the age range of 61-70 years in our study. Female preponderance [62.5%] was seen.
11. The maximum numbers of cases of NENs in pancreas were of neuroendocrine grade 1 [6]. Two primary cases of neuroendocrine neoplasms of liver were seen in present study.
12. Other organs such as adrenal, breast, prostrate, thyroid etc. were also involved by neuroendocrine neoplasms.

To conclude neuroendocrine neoplasms are distinct neoplasms that differ clinically, histopathologically and immunohistochemically from other tumors. So, correlation of histopathological diagnosis with immunohistochemistry is important to give a definitive diagnosis as the prognosis and treatment of these tumors differ from other tumors.

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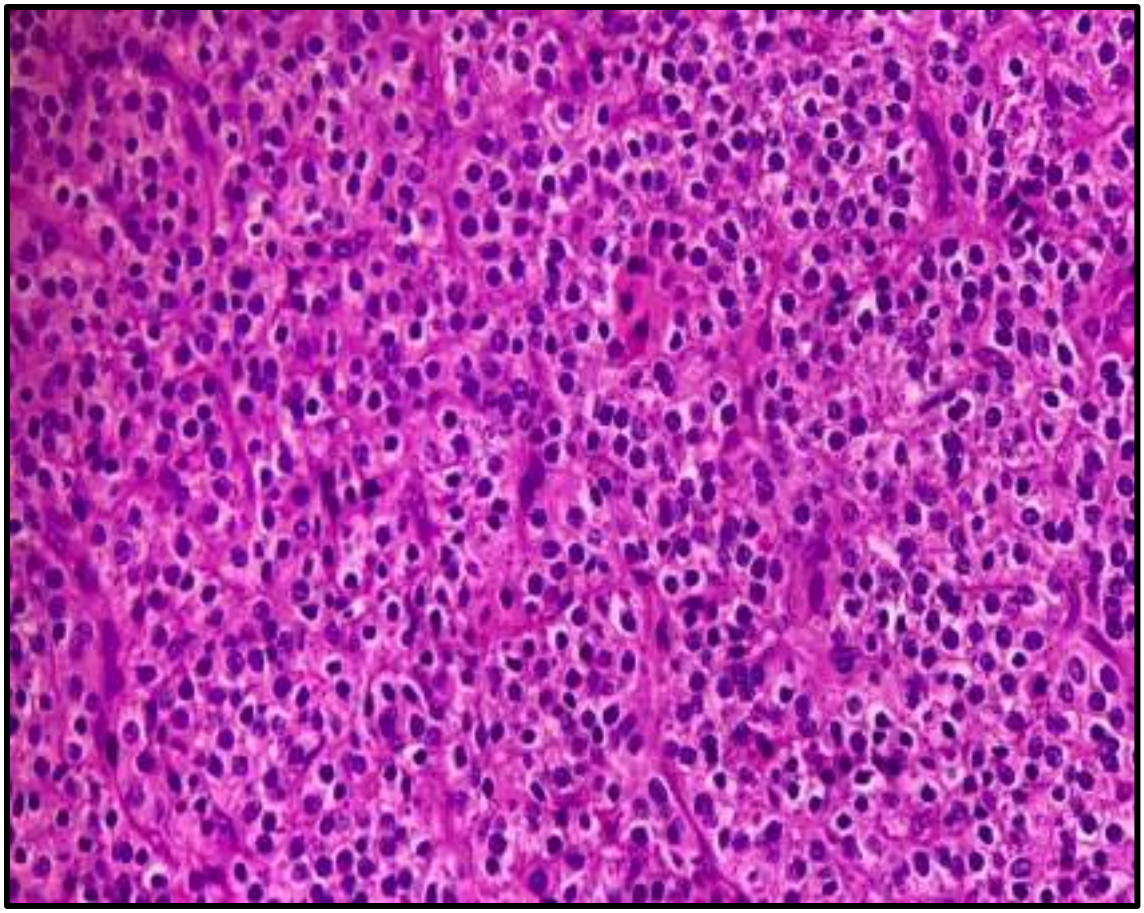
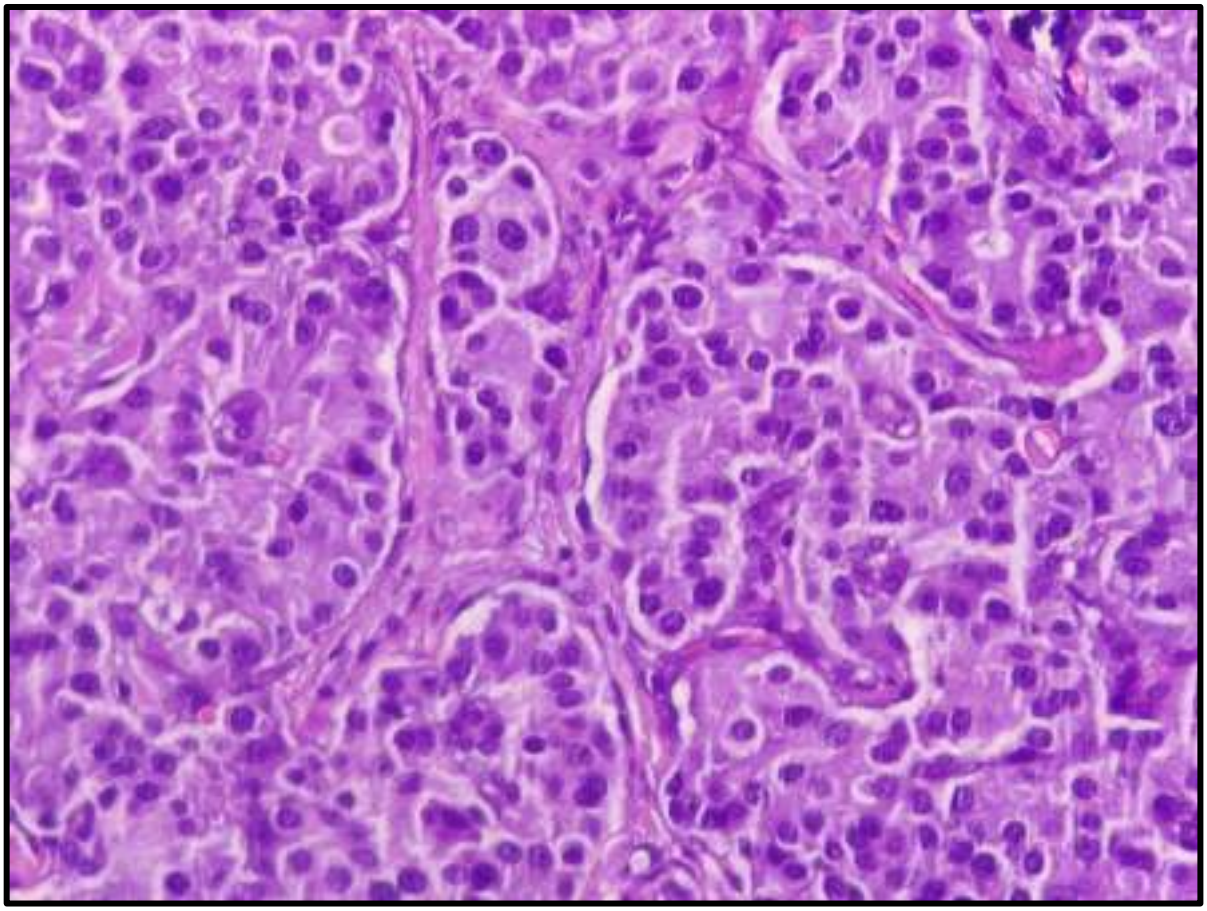
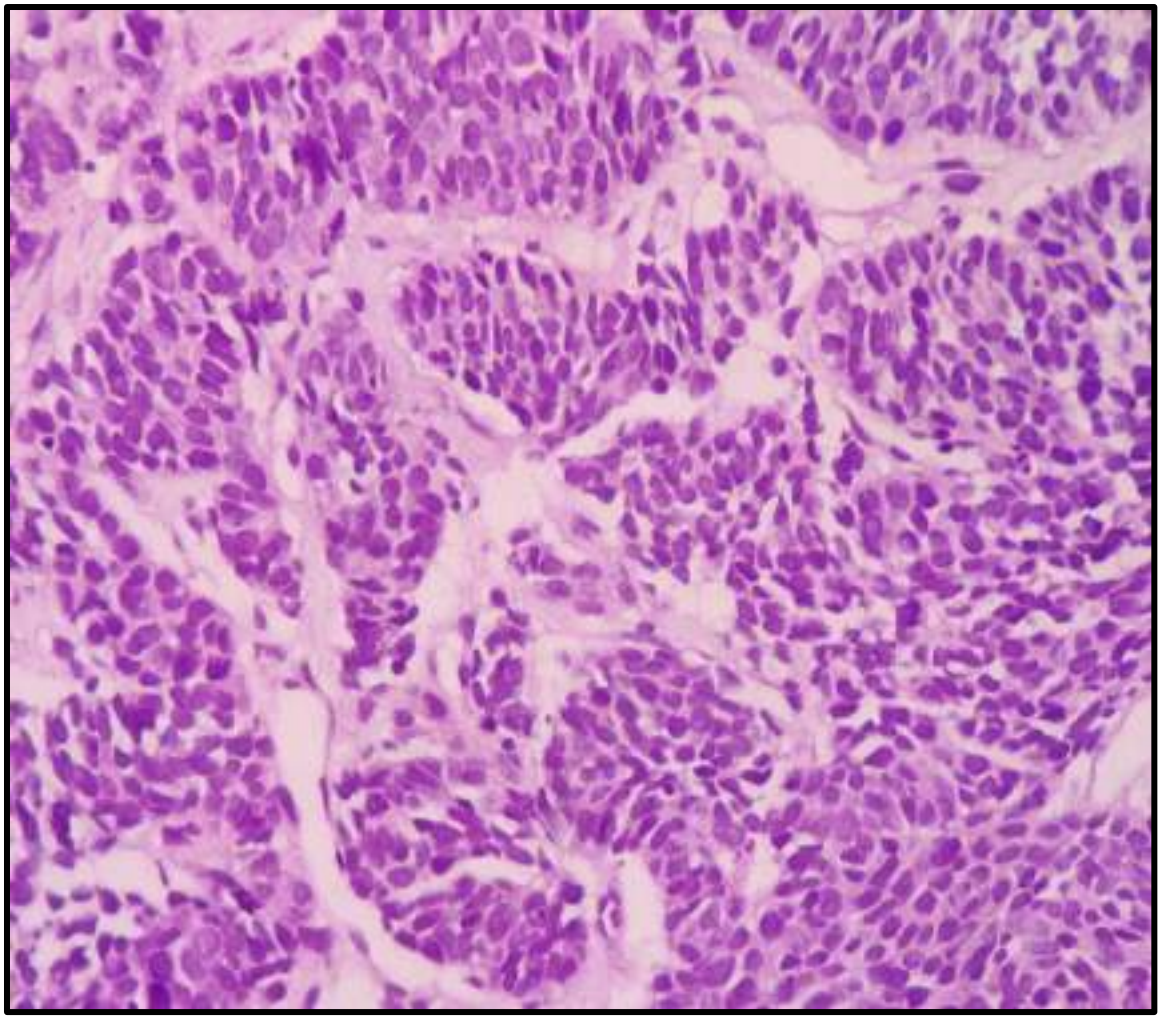


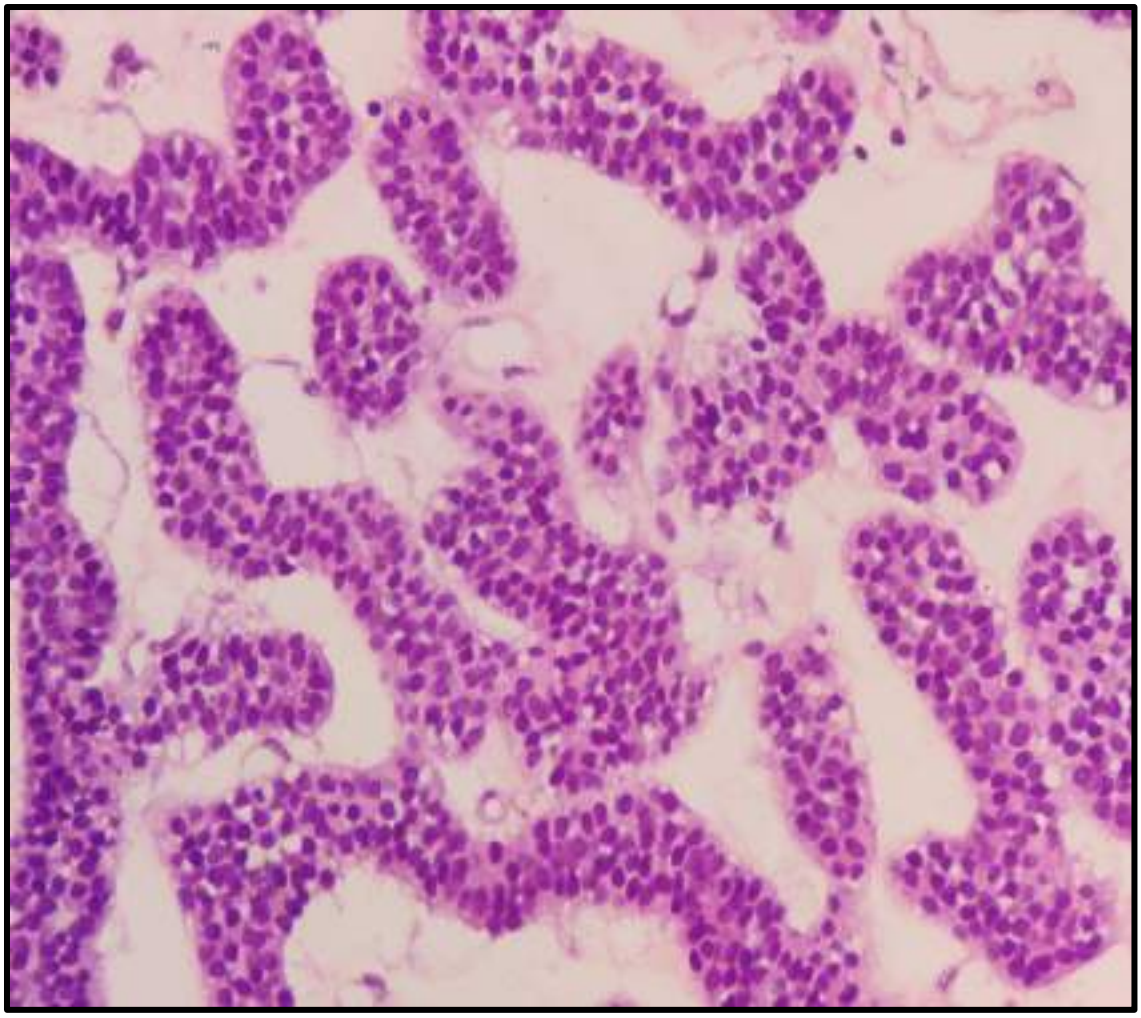
Picture 1– Neuroendocrine Tumor of small intestine,
photograph showing zellballen pattern of tumor cells (H&E, 400X)



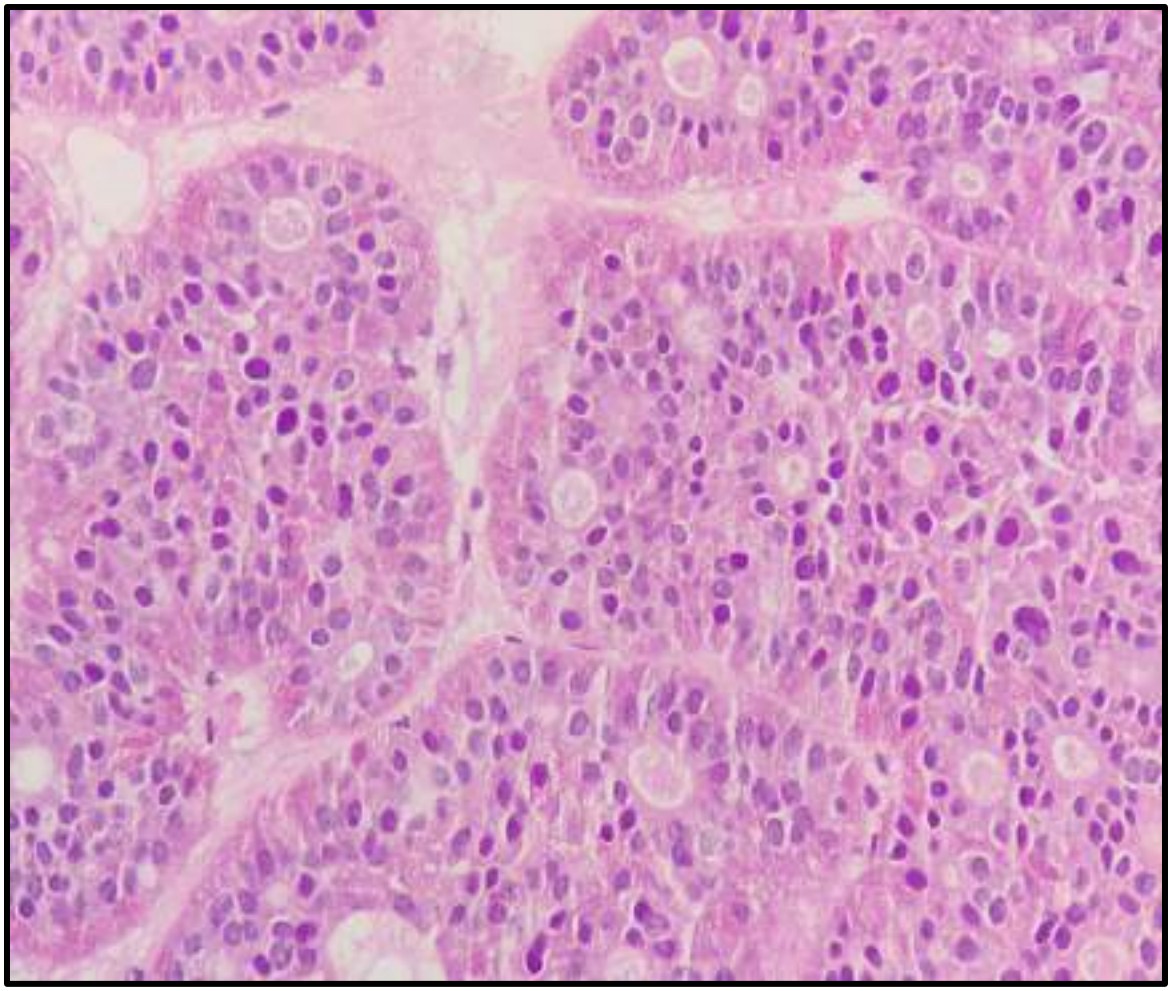
Picture 2- Neuroendocrine Tumor of pancreas, organoid pattern of tumor cells (H&E, 400X)



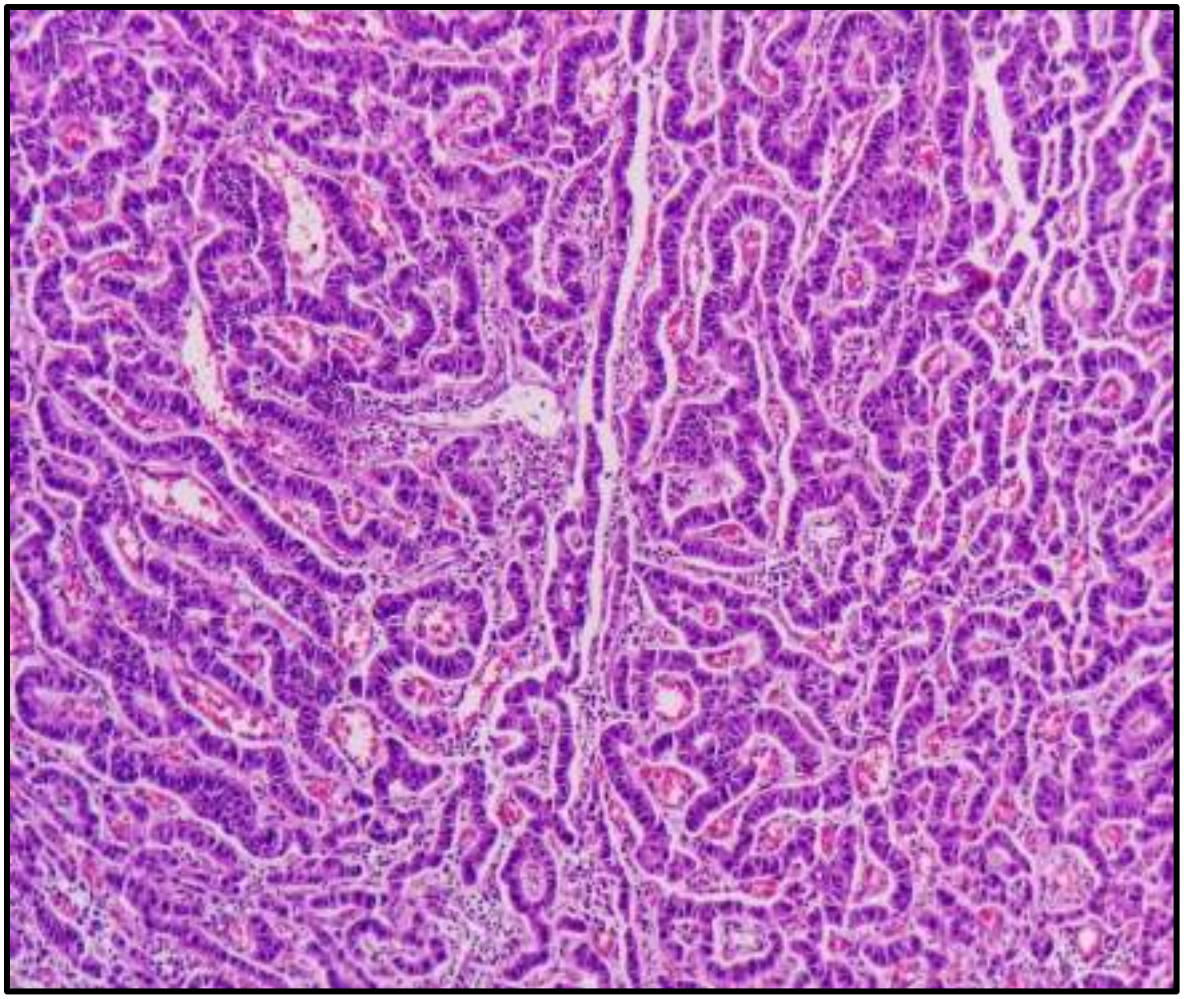
Picture 3– Neuroendocrine Tumor of small Intestine, nesting pattern of tumor cells (H&E, 400X)



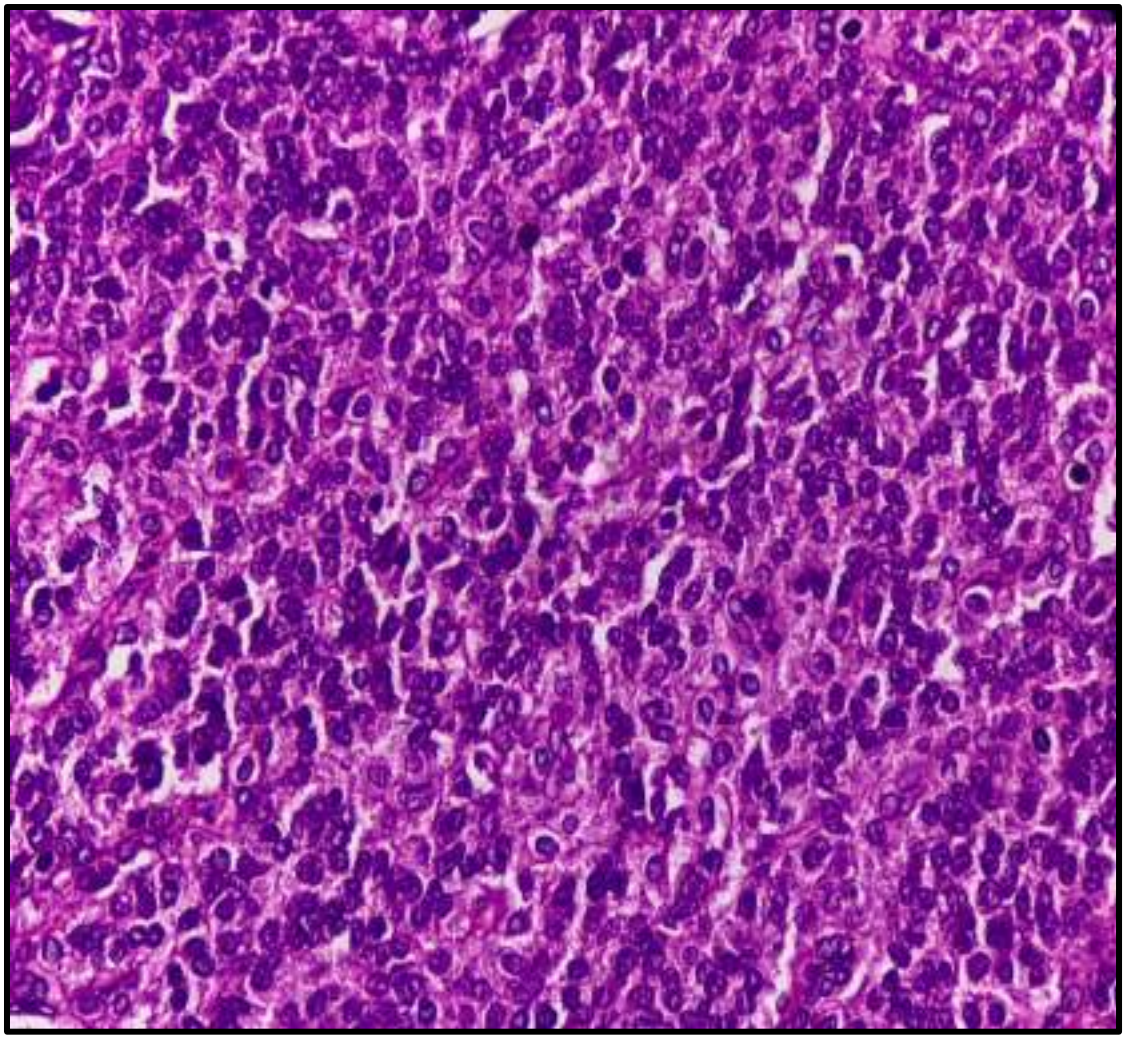
Picture 4 – Neuroendocrine Tumor in liver, trabecular arrangement of tumor cells (H&E, 400X)



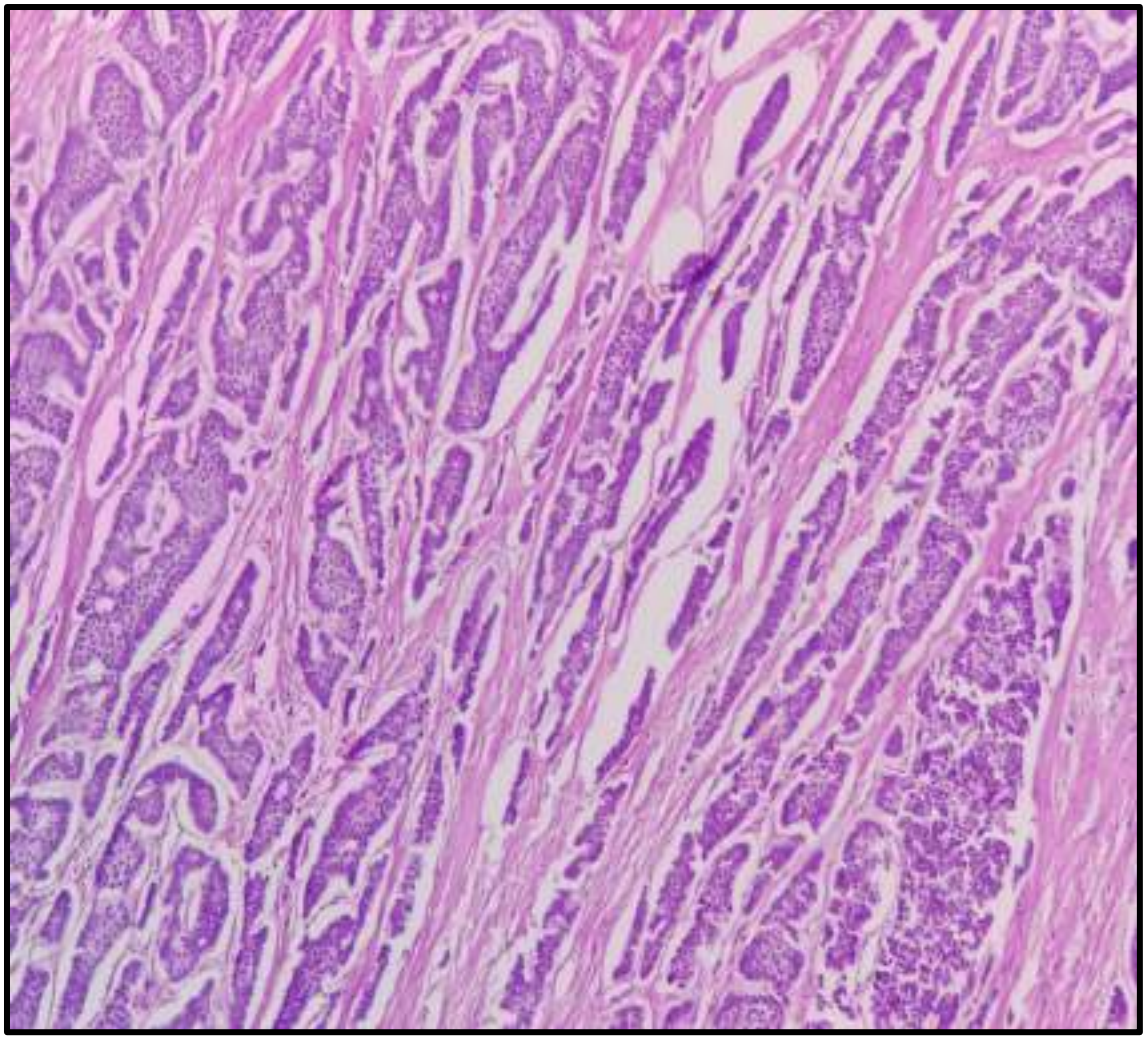
Picture 5- Neuroendocrine Tumor in liver, glandular architecture of tumor cells (H&E, 400X)



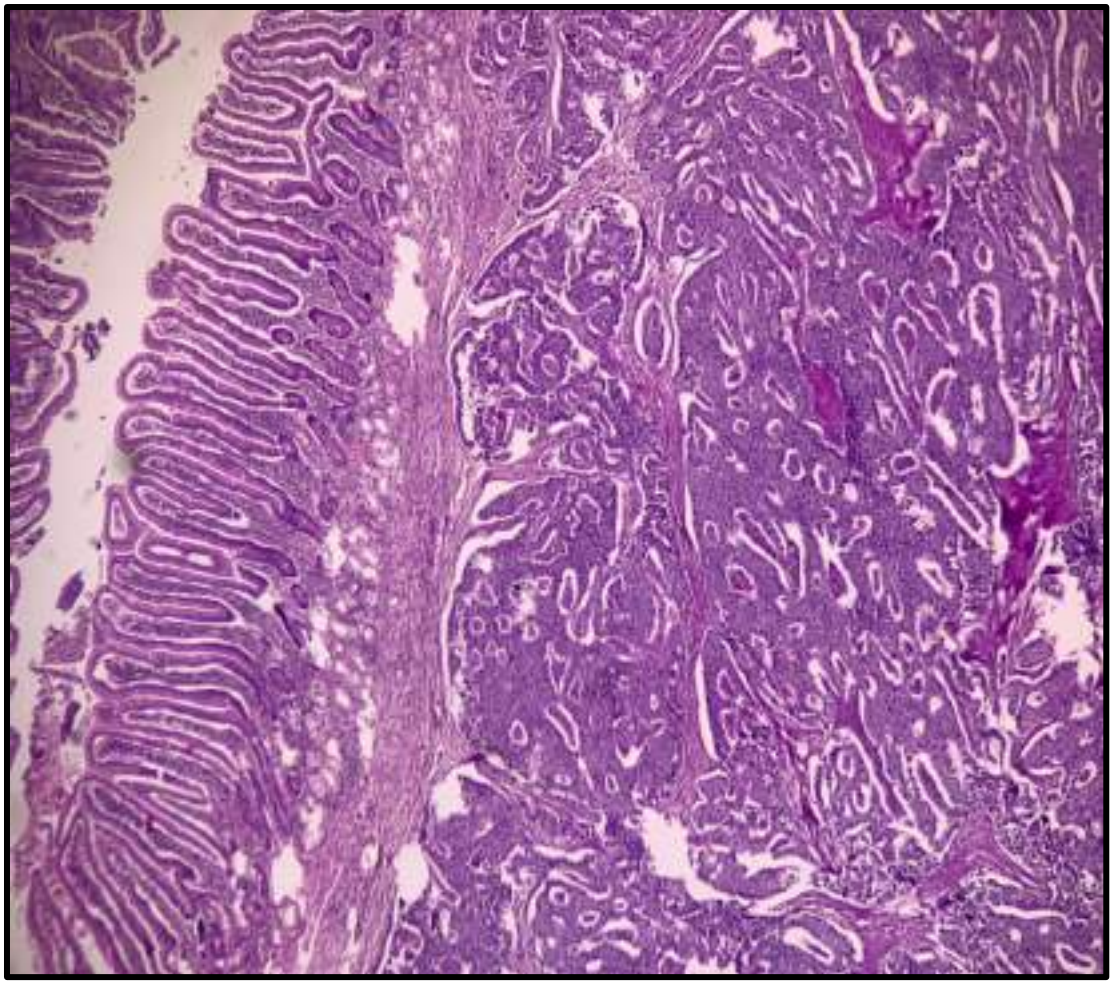
Picture 6- Neuroendocrine Tumor of stomach, gyriform pattern of tumor cells (H&E, 400X)



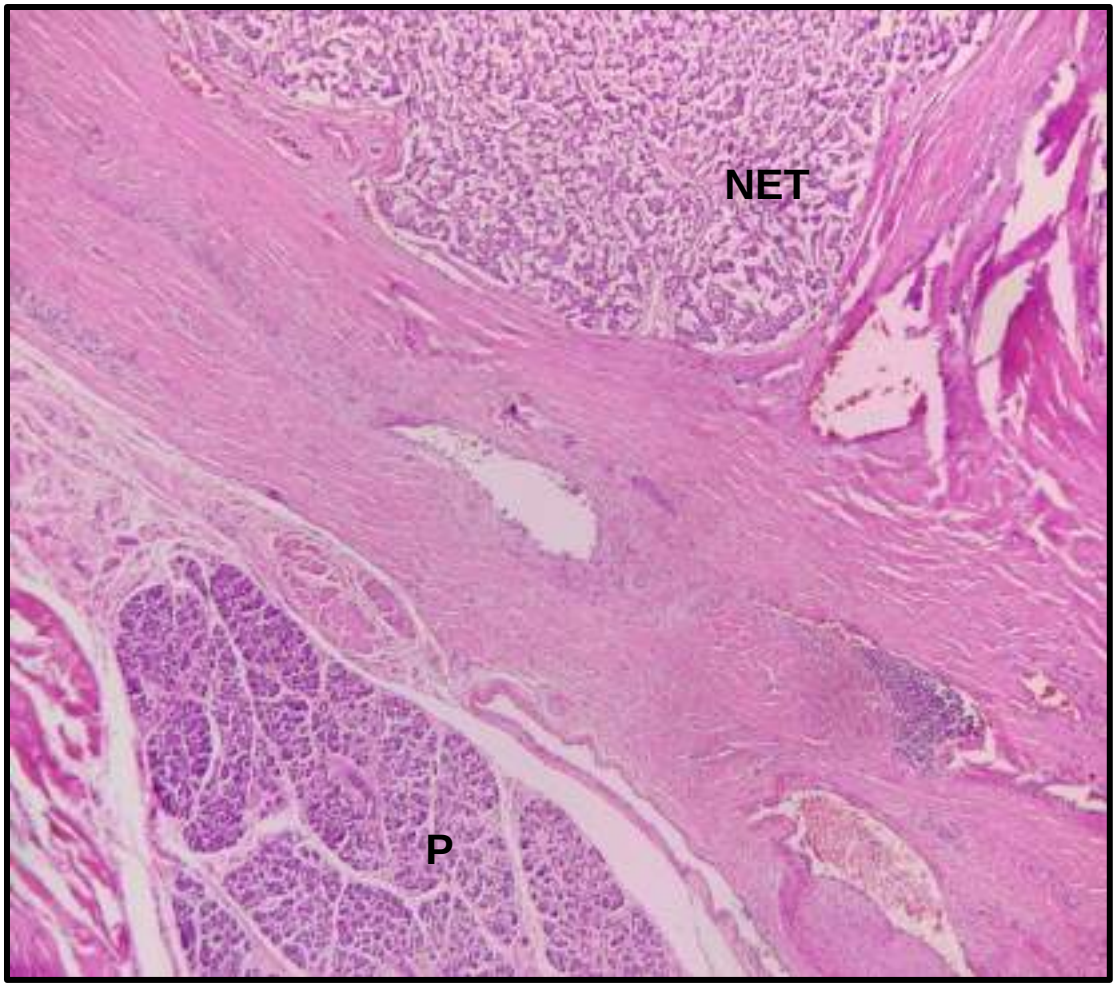
Picture 7– Neuroendocrine Tumor of small intestine, sheeting pattern of tumor cells (H&E, 400X)



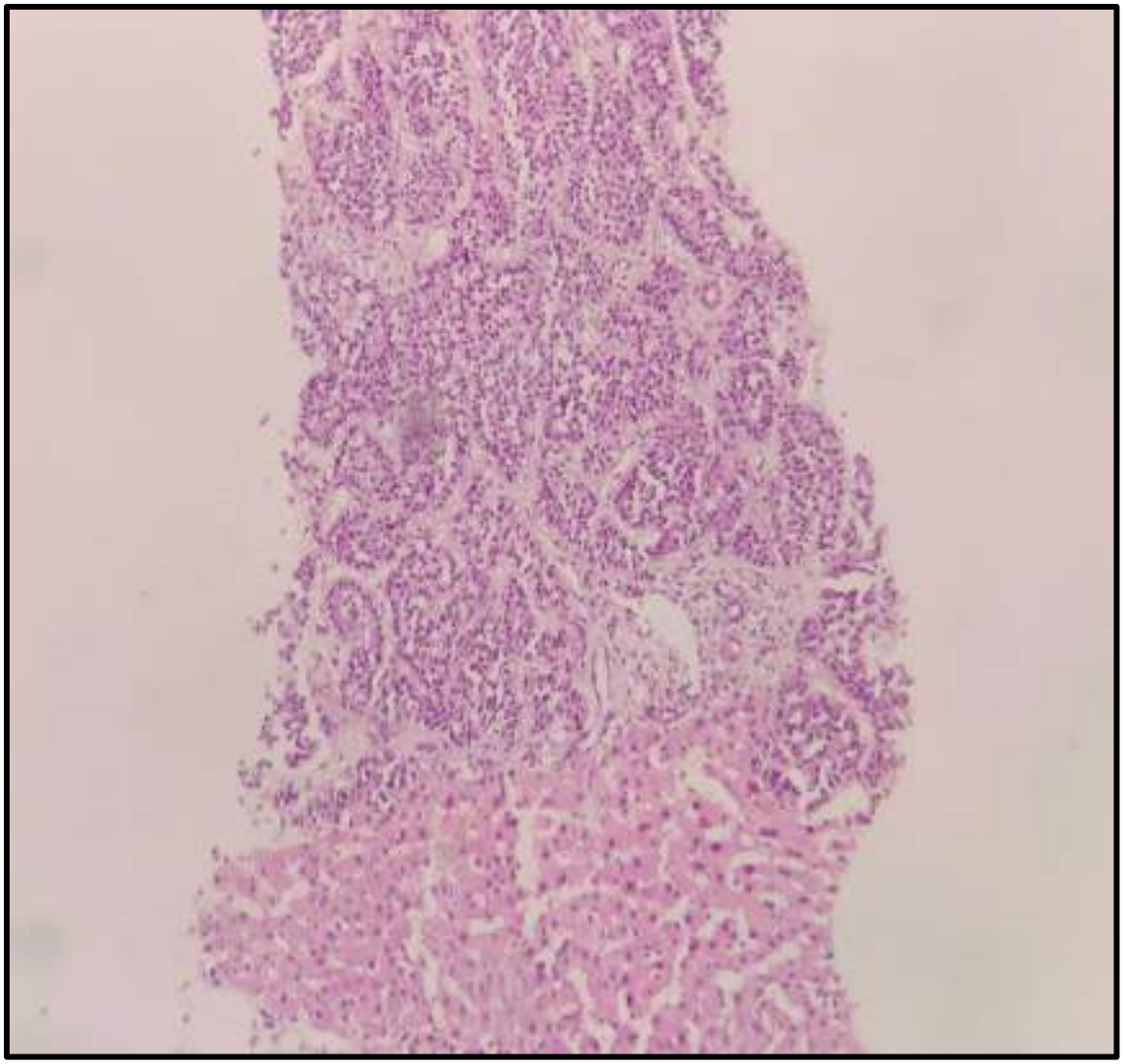
Picture 8– Neuroendocrine Tumor cells in mixed pattern i.e. cords, trabculae & nests (H&E, 100X)



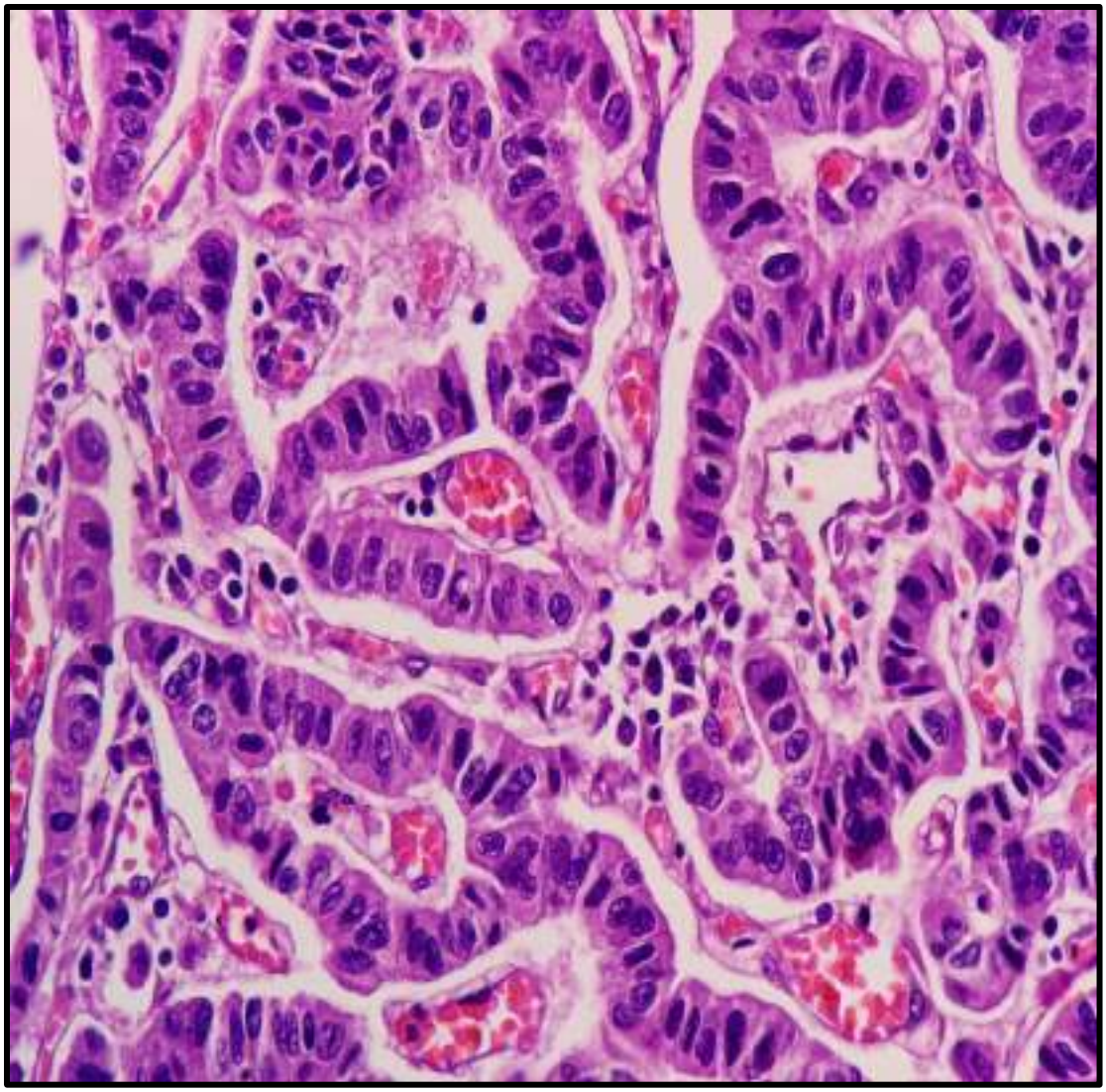
Picture 9- Neuroendocrine Tumor arising in submucosa of small intestine (H&E, 100X)



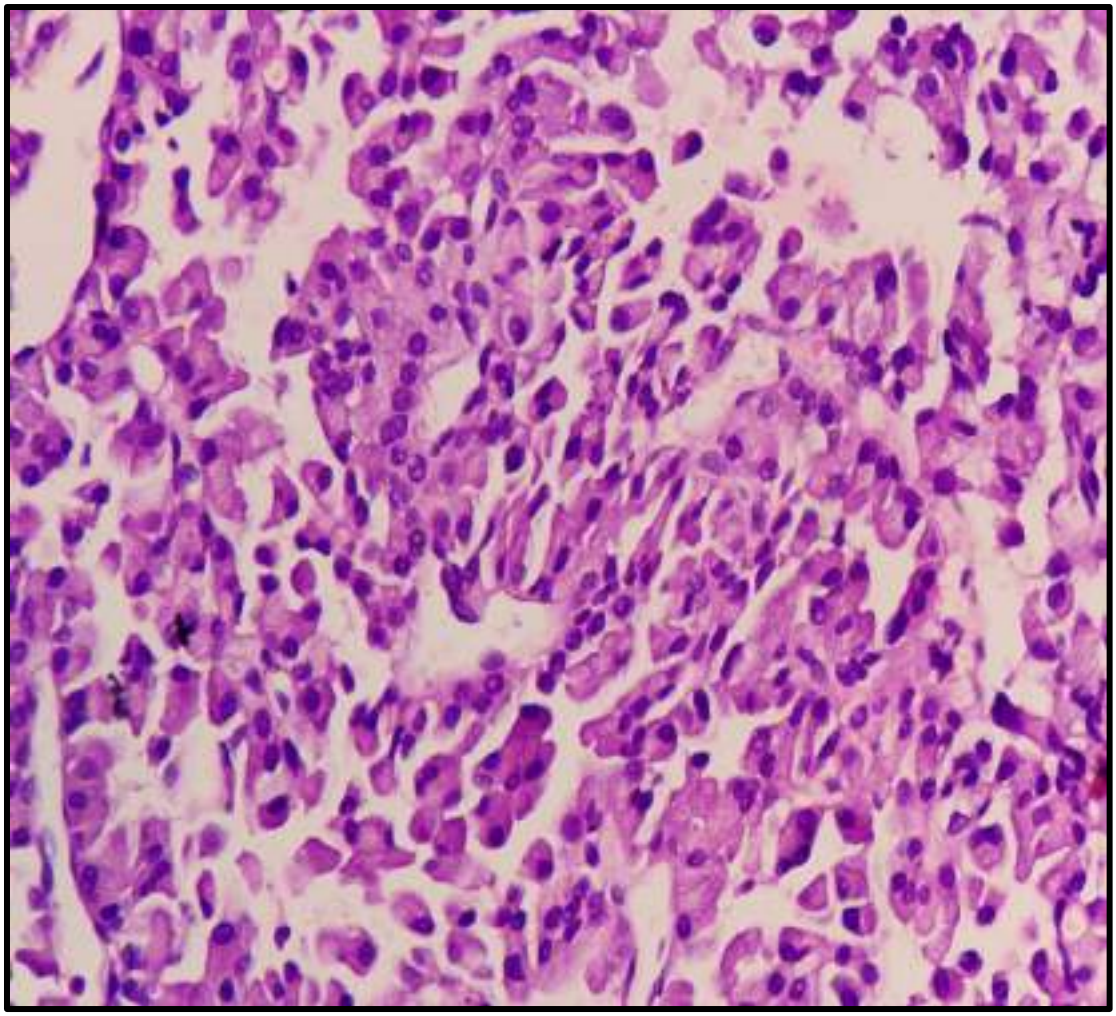
Picture 10– Neuroendocrine Tumor cells (upper half) arranged in nests (H&E, 100X) (NET- Neuroendocrine Tumor, P- Pancreas)



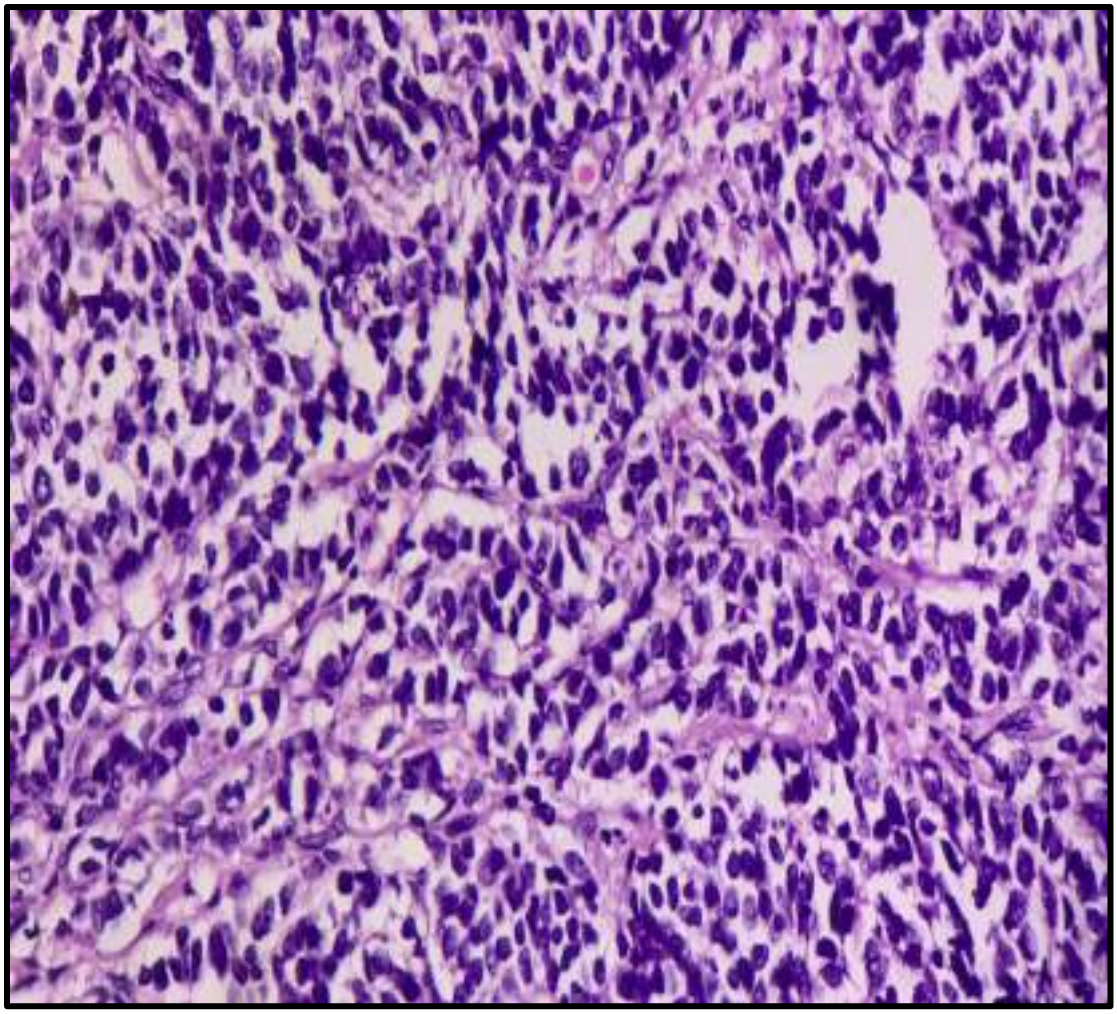
Picture 11– Neuroendocrine Tumor -Liver (H&E, 100X)



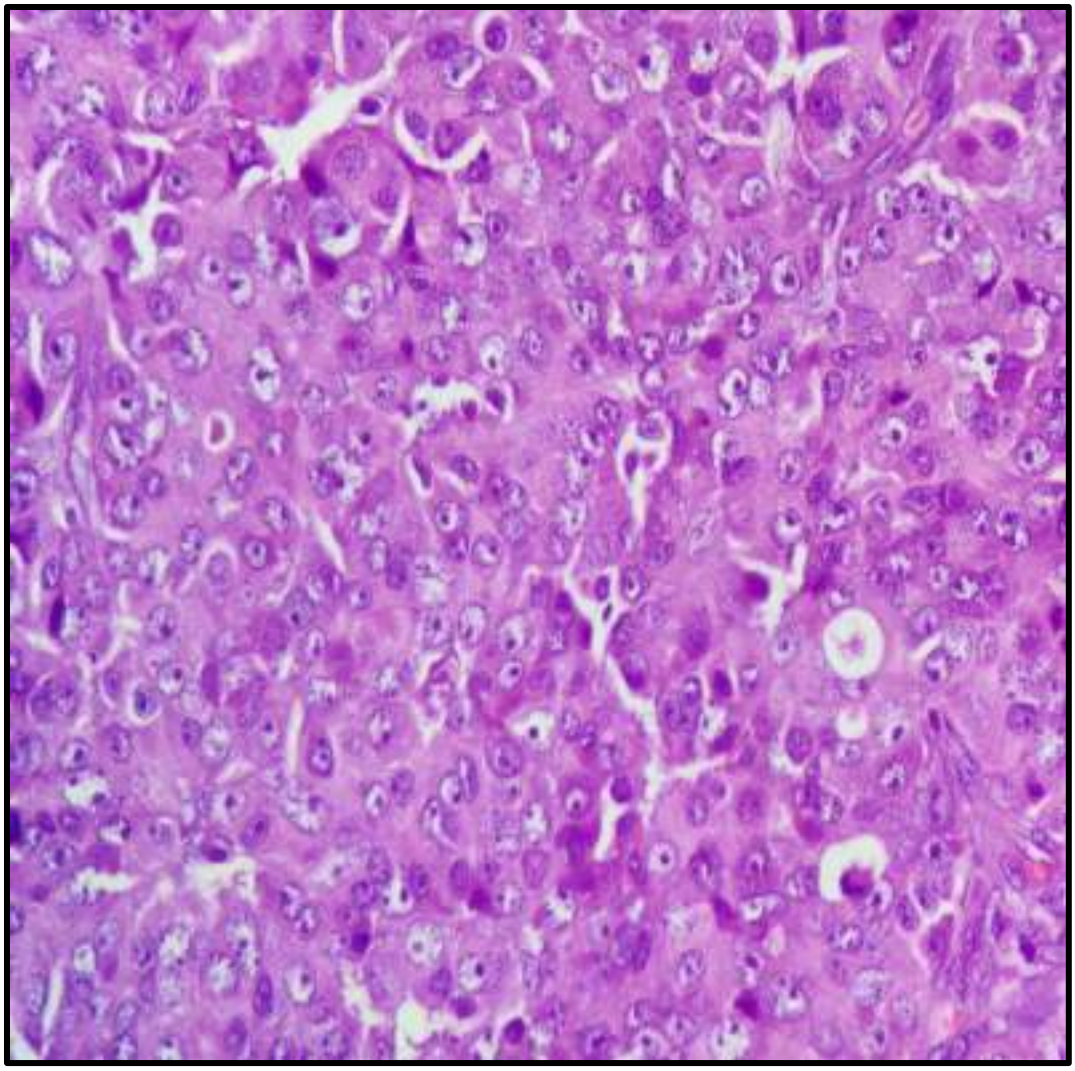
Picture 12– Well differentiated Neuroendocrine Tumor- Small Intestine (H&E, 400X)



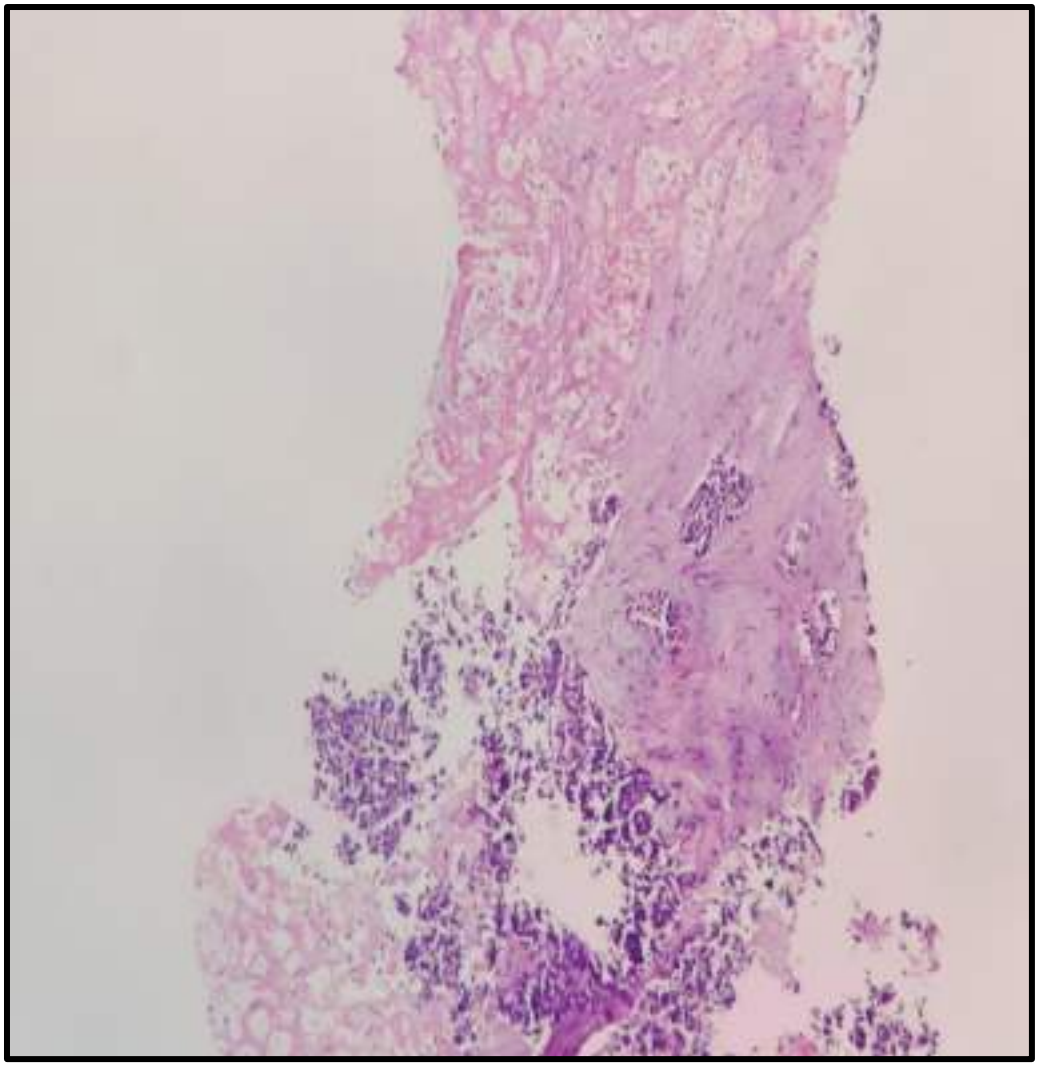
Picture 13 - Well differentiated Neuroendocrine Tumor of pancreas, having plasmacytoid morphology of the cells (H&E, 400X)



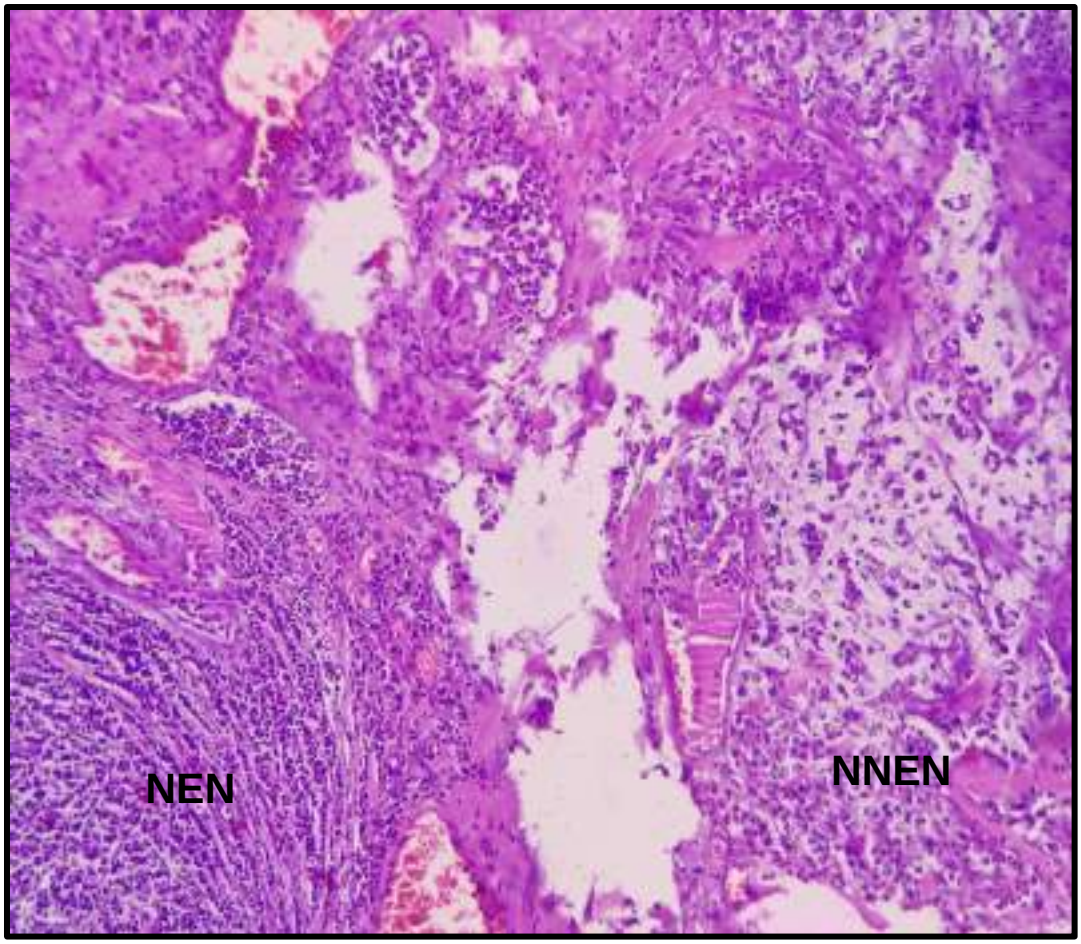
Picture 14– Small cell carcinoma of lung, sheeting pattern of tumor cells with hyperchromatic nuclei and scant amount of cytoplasm (H&E, 400X)



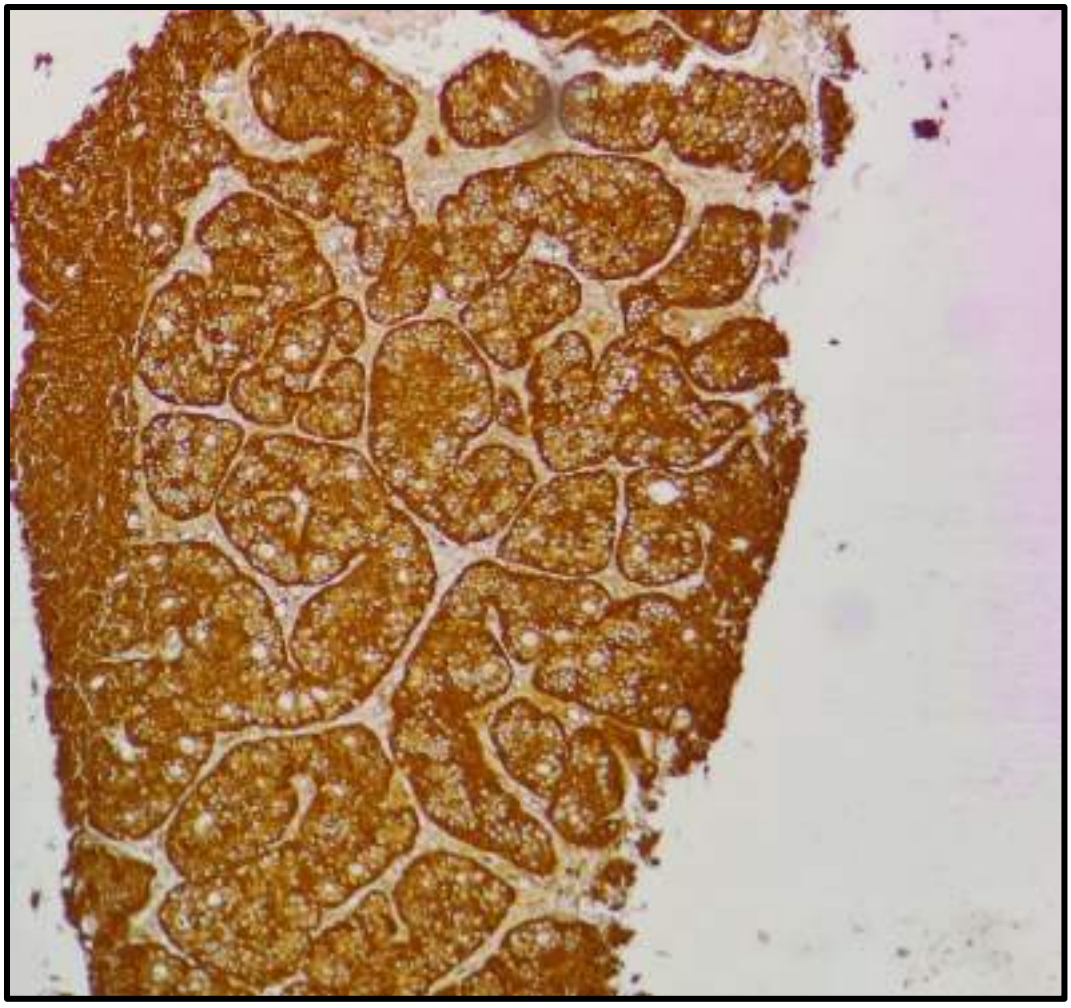
Picture 15- Large cell neuroendocrine carcinoma of small intestine, tumor cells having vesicular nuclei with prominent nucleoli and abundant cytoplasm (H&E, 400X)



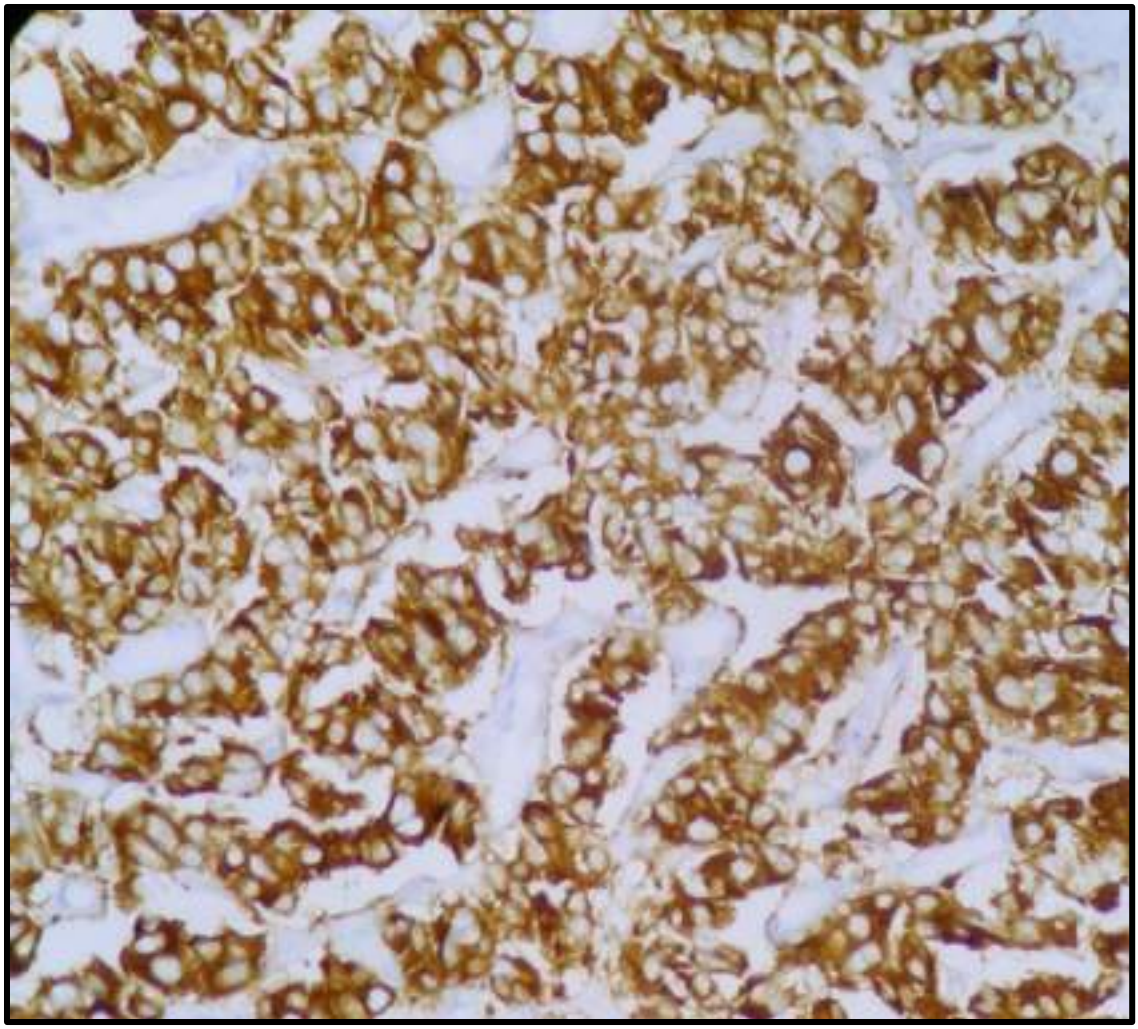
Picture 16- Neuroendocrine carcinoma- liver with extensive areas of necrosis (H&E, 100X)



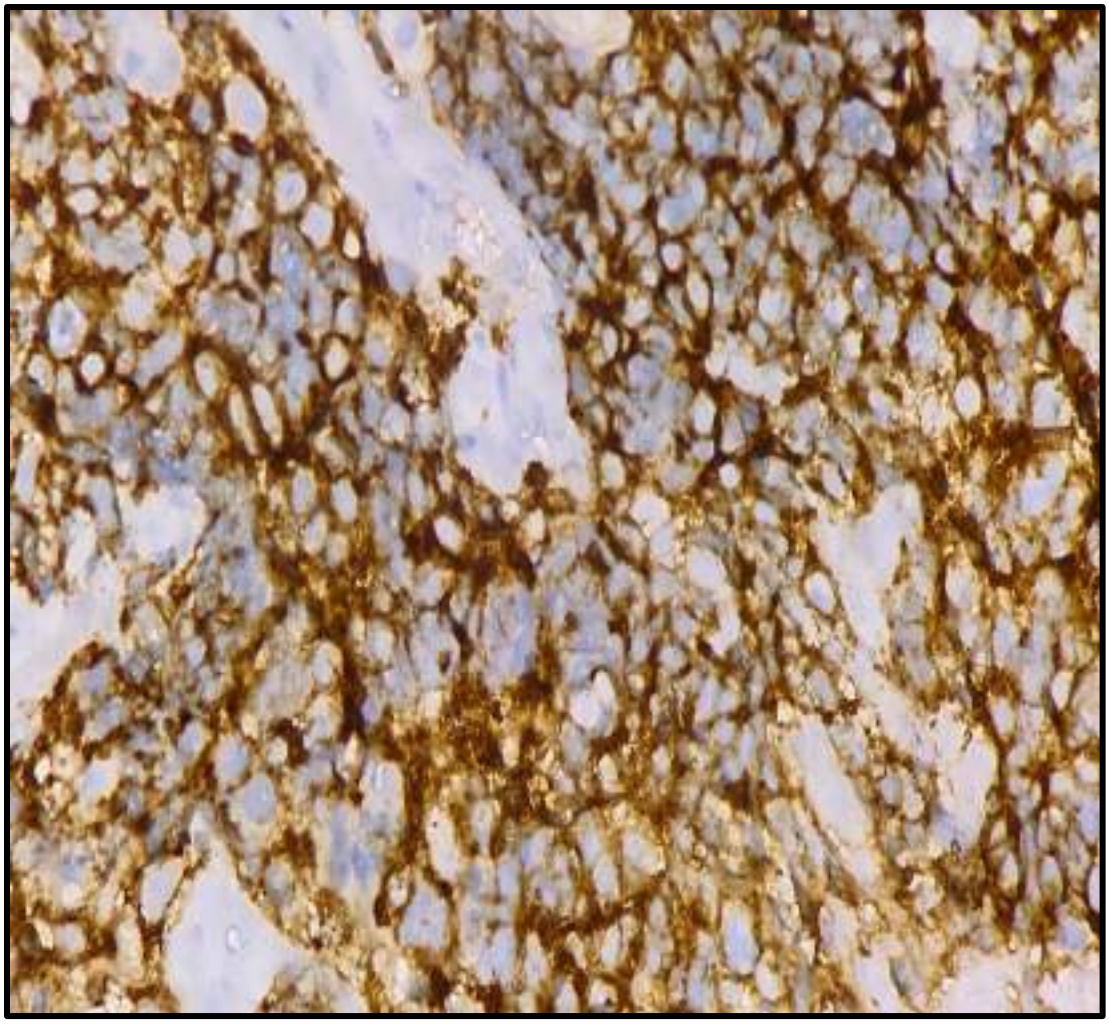
Picture 17– Photomicrograph showing Mixed Neuoroendocrine– Non Neuroendocrine Neoplasm of Small Intestine (H&E, 400X) (NNEN- Non Neuroendocrine Neoplasm, NEN – Neuroendocrine Neoplasm)



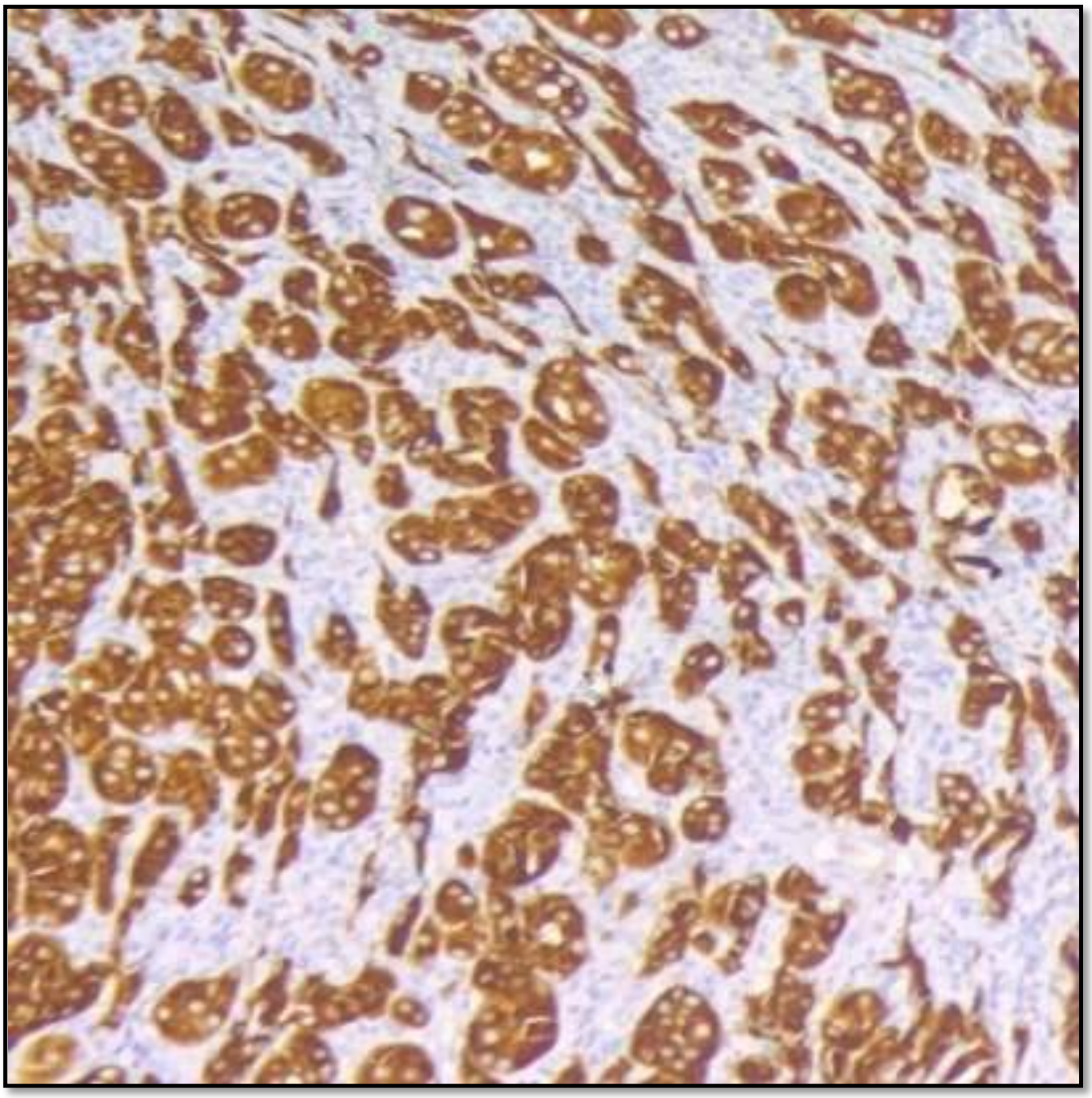
Picture 18- Neuroendocrine Tumor - Cells showing diffuse strong cytoplasmic immunoreactivity of Synaptophysin (100X)



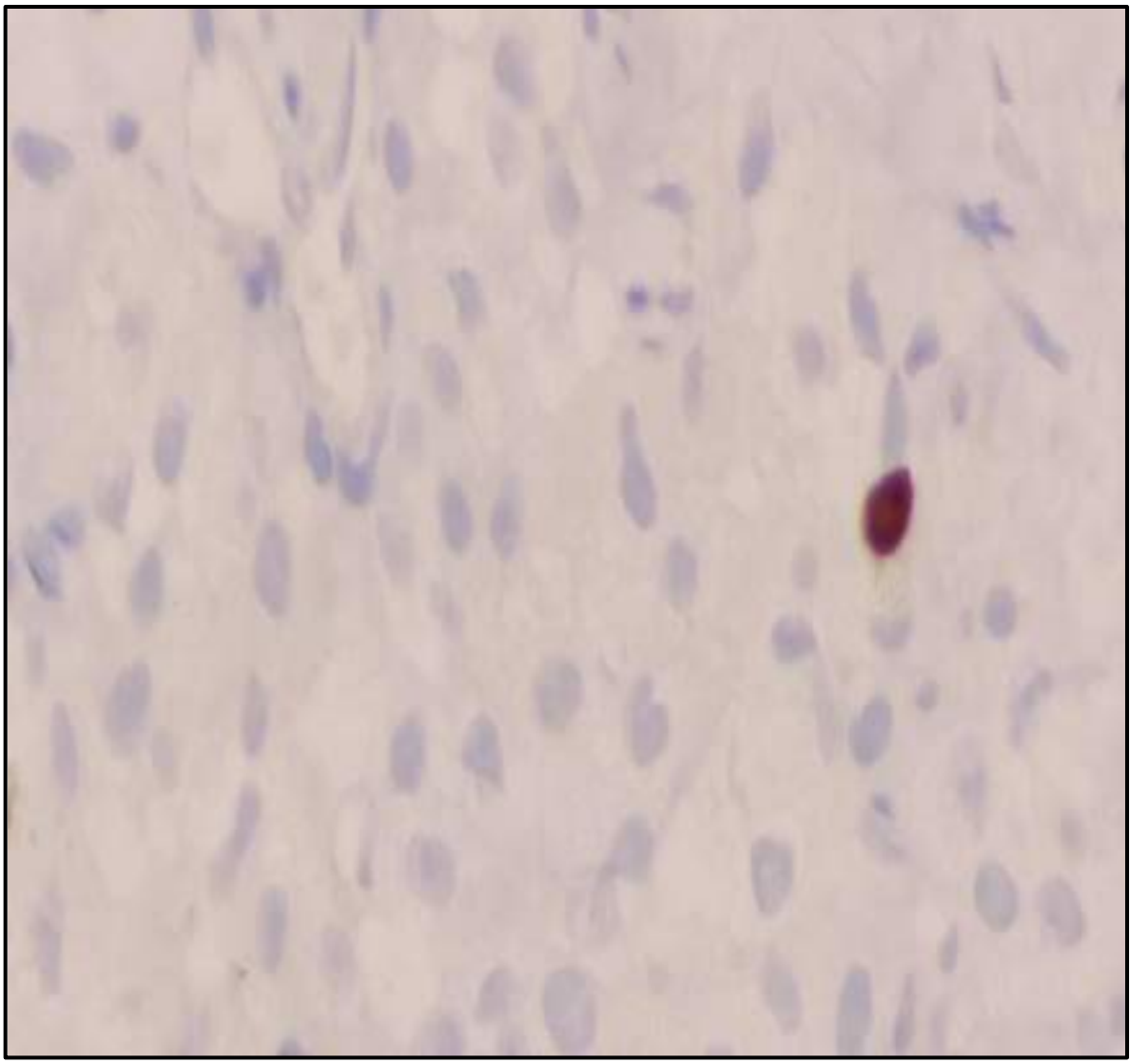
Picture 19– Neuroendocrine Tumor - Cells showing diffuse strong cytoplasmic immunoreactivity of Chromogranin A (400X)



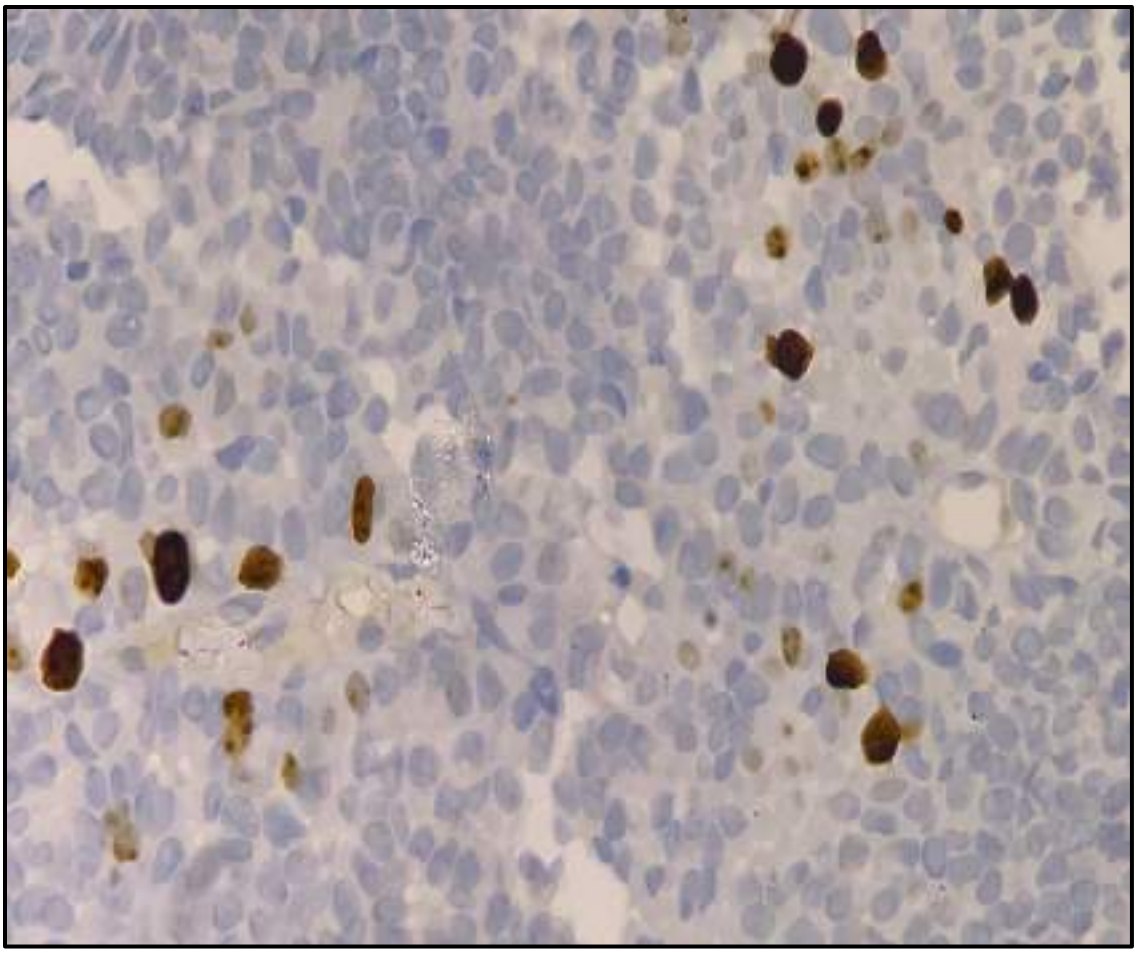
Picture 20– Neuroendocrine Tumor - Cells showing diffuse strong membranous immunoreactivity of CD56 (400X)



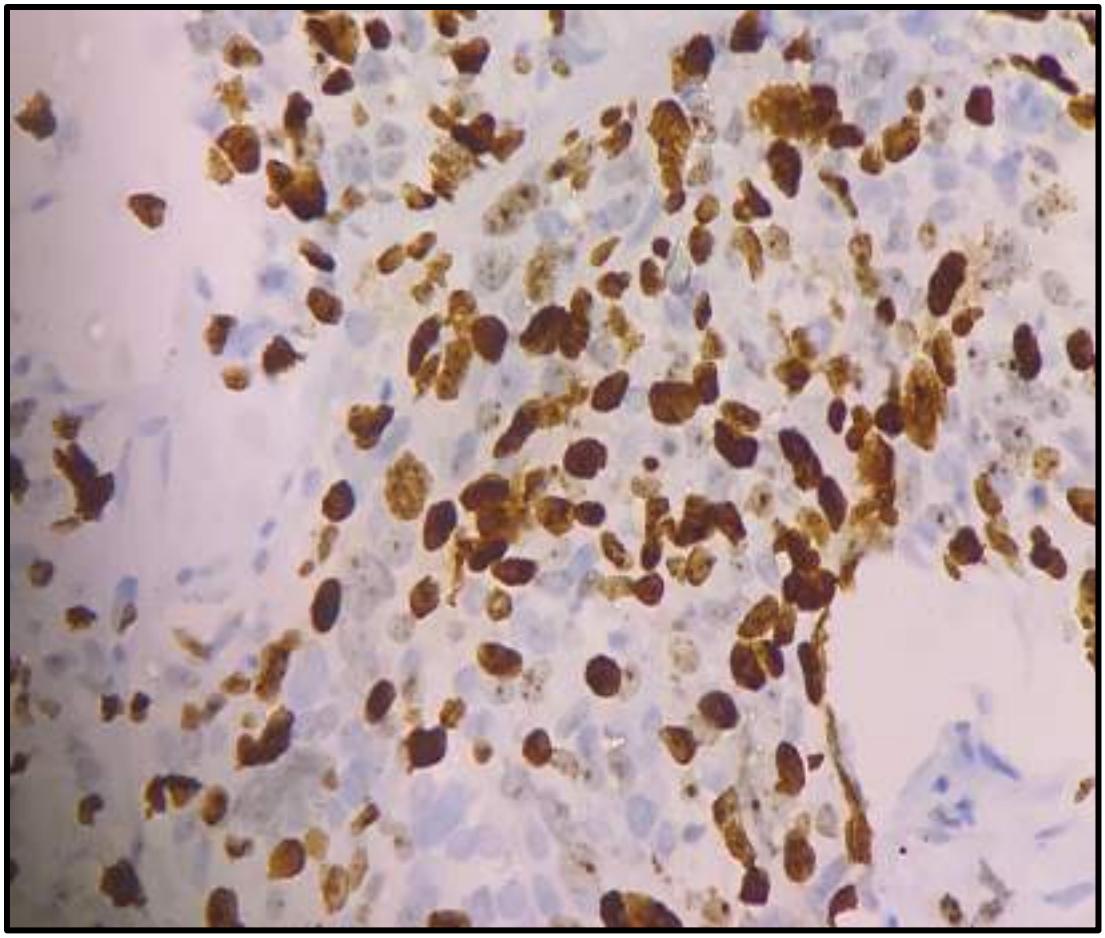
Picture 21- Neuroendocrine Tumor - Cells showing diffuse strong cytoplasmic immunoreactivity of NSE (100X)



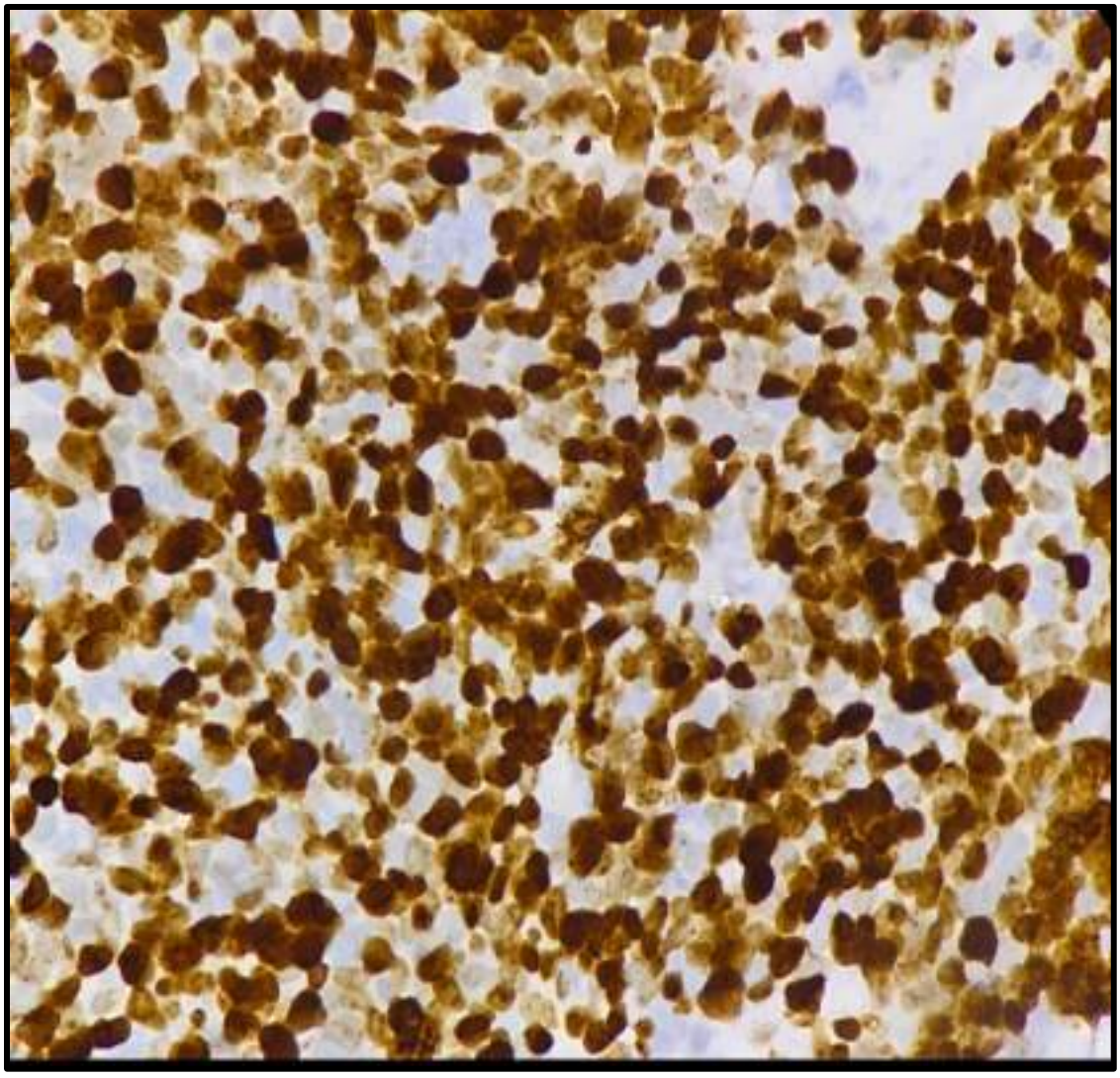
Picture 22– Well Differentiated Neuroendocrine Tumor- Ki-67 proliferation index less than 3% (400X)



Picture 23– Well Differentiated Neuroendocrine Tumor- Ki-67 proliferation index between 3-20% (400X)



Picture 24– Photomicrograph of Neuroendocrine Tumor- Ki-67 proliferation index between 21- 50% (400X)



Picture 25– Neuroendcorine Carcinoma - Ki-67 proliferation index>90% (400X)

S.NO	Name	Age	Sex	Date	MRD-NO	Chief complaints	Radiology -CT/MRI/US	Location	Histo -no	Architecture						Cell Size	Pleomorphic	Nuclear Features	Spindling	Nucleoli	Cytoplasm	Mitosis	Necrosis	IHC -NO	Chromogranin			Synaptophysin			
										Glands	Nests	Trabecular	Zell ballen	Sheets	Others									Intensity	location	Distrubtion	Intensity	location	Distrubtion	Intensity
1	BK	58	F	7.7.15	101945	Loss of appetite ,loss of weight	Not available	Small intestine	15-5950	+	+	-	-	-	-	small	M	round nuclei with stippled chromatin	-	-	moderate amount of cytoplasm	1/10hpf	not seen	15-670-671	3+	C	D	3+	C	D	
2	NR	58	M	23.7.15	132436	Pain abdomen	Mass in left lobe of lung	Lymph Node	15-7227	-	+	-	-	-	-	smal	P	round hyperchromatic nuclei	+	-	scant amount of cytoplasm	12/10hpf	not seen	15-737	3+	C	D	3+	C	D	
3	JK	58	F	3.8.15	139478	Breathlesness	Not available	Lung	15-7607	-	-	-	-	+	-	small	P	round hyperchromatic nuclei	+	-	scant amount of cytoplasm	15/10hpf	focally present	15-741,743	3+	C	F	3+	C	D	
4	SS	45	M	29.9.15	182353	Diarrhoas,loss of weight	Not available	Small intestine	15-9601	-	+	-	+	-	-	small	M	round vesicular nuclei	-	-	moderate amount of cytoplasm	2/10hpf	not seen	15-917	3+	C	D	not done			
5	SS	44	F	30.9.15	169051	Loss of appetite ,loss of weight	Not available	Ampulla	15-9536	-	-	+	+	-	-	small	M	round nuclei with stippled chromatin	-	-	moderate amount of cytoplasm	2/10hpf	not seen	15-923	3+	C	D	3+	C	D	
6	PL	66	F	5.10.15	238007	Jaundice,loss of appetite	Not available	Gall bladder	15-11573	-	+	-	-	+	-	large	P	round vesicular nuclei	-	+	moderate amount of cytoplasm	22/10hpf	not seen	15-1060	3+	C	D	3+	C	D	
7	BR	72	M	20.5.16	102093	loss of appetite ,loss of weight	not available	Large intestine	16-4693	-	+	-	-	-	-	small	M	round vesicular nuclei	-	-	moderate amount of cytoplasm	4/10hpf	not seen	16-422,423	3+	C	D	3+	C	D	
8	DS	60	M	28.5.16	235917	Difficulty in swallowing	Solid lesion in left lobe of lung	Lymph node	16-5520	-	+	-	-	+	cords	large	P	round vesicular nuclei	-	+	moderate amount of cytoplasm	25/10hpf	focally present	16-449,450	not done			3+	C	F	3+
9	HS	68	M	15.7.16	170760	loss of appetite ,loss of weight	not available	Lymph node	16-6650	-	+	-	-	-	-	small	M	round nuclei with stippled chromatin	-	-	moderate amount of cytoplasm	1/10hpf	not seen	16-450	3+	C	D	3+	C	D	
10	AD	48	M	18.7.16	60140	pain abdomen	not available	Small intestine	16-6652	-	+	-	-	-	-	small	M	round vesicular nuclei	-	-	moderate amount of cytoplasm	1/10hpf	not seen	16-611,612	3+	C	D	Not done			3+
11	ICJ	70	M	10.8.16	128007	loss of appetite ,loss of weight	not available	Liver	16-8171	-	+	-	-	+	-	large	P	round vesicular nuclei	-	+	moderate amount of cytoplasm	22/10hpf	focally present	16-613	3+	C	D	Not done			
12	GL	64	M	9.11.16	153376	diarrohea	not available	Small intestine	16-11001	-	-	-	+	-	acinar	small	M	round vesicular nuclei	-	-	moderate amount of cytoplasm	1/10hpf	not seen	16-1047,1049	3+	C	D	Not done			
13	KM	50	F	13.12.16	149000	loss of appetite ,loss of weight	not available	Small intestine	16-12487	-	+	-	+	-	-	small	M	round nuclei with stippled chromatin	-	-	moderate amount of cytoplasm	2/10hpf	not seen	16-1146-1150	3+	C	D	Not done			
14	DK	58	F	13.12.16	143165	pain abdomen	enhacing Soft tissue lesion with areas of necrosis	Lymph node	16-12455	-	+	-	-	-	-	small	M	round nuclei with stippled chromatin	-	-	abundant amount of cytoplasm	2/10hpf	not seen	16-1137,1139	3+	C	D	3+	C	D	
15	KK	30	F	20.1.17	155010	Pain abdomen ,hypertension	Not available	Adrenal	17-214	-	+	-	-	-	-	small	M	round vesicular nuclei	-	-	moderate amount of cytoplasm	2/10hpf	not seen	17-52	3+	C	D	3+	C	F	
16	RK	60	M	11.2.17	178785	Breathlessness	Lesion seen in right lobe of lung	Lung	17-1015	+	+	-	-	-	islands	large	P	round vesicular nuclei	-	+	scant amount of cytoplasm	25/10hpf	present	17-138-145	3+	C	F	not done			
17	SW	24	F	29.3.17	219821	Loss of appetite ,loss of weight	Not available	Breast	17-2603	-	+	+	-	-	-	small	M	round vesicular nuclei	-	-	scant amount of cytoplasm	8/10hpf	focally present	17-300	3+	C	F	not done			
18	MS	45	M	13.4.17	233448	Chest pain	lesion seen in right lobe of lung	Lymph node	17-3229	-	+	-	-	-	islands	small	M	round vesicular nuclei	-	-	moderate amount of cytoplasm	4/10hpf	not seen	17-321	3+	C	D	3+	C	D	
19	JS	65	M	24.4.17	110144	Breathlessness	solid mass in left upper lobe of lung	Lung	17-3577	-	+	-	-	-	-	small	P	round hyperchromatic nuclei	+	-	scant amount of cytoplasm	12/10hpf	not seen	17-342	3+	C	D	3+	C	D	
20	GK	62	F	8.5.17	249247	Loss of appetite ,loss of weight	Consolidating mass in left lobe of lung	Liver	17-4185	-	+	-	-	-	-	small	M	round nuclei with stippled chromatin	-	-	moderate amount of cytoplasm	2/10hpf	not seen	17-367	3+	C	D	3+	C	D	
21	GK	55	F	30.5.17	214931	pain abdomen	Not available	Lymph node	17-4941	-	-	-	-	+	-	small	P	round vesicular nuclei	-	-	moderate amount of cytoplasm	8/10hpf	not seen	17-388	3+	C	F	not done			
22	DK	40	M	30.5.17	270218	Loss of appetite ,loss of weight	a hypoechoeic area seen in quadrant 4	Liver	17-5662	-	+	+	-	-	-	large	P	round vesicular nuclei	-	+	moderate amount of cytoplasm	22/10hpf	focally present	17-405	3+	C	D	3+	C	D	
23	KS	34	F	5.6.17	152513	diarhoea,weight loss	Not available	Small intestine	17-5407	-	+	-	-	-	-	small	M	round vesicular nuclei	-	-	moderate amount of cytoplasm	4/10hpf	not seen	17-407	3+	C	D	3+	C	D	
24	AK	60	F	19.6.17	273157	flushing ,hypertension	Not available	Adrenal	17-6240	-	+	-	+	-	-	small	M	round vesicular nuclei	-	-	moderate amount of cytoplasm	5/10hpf	not seen	17-444	3+	C	F	3+	C	F	
25	KD	70	F	27.7.17	300318	pain abdomen	Not available	Small intestine	17-7803	-	+	-	-	-	-	small	M	round vesicular nuclei	-	-	moderate amount of cytoplasm	1/10hpf	not seen	17-515	3+	C	D	3+	C	D	
26	SK	59	F	4.8.17	310457	Loss of appetite ,loss of weight	Not available	Breast	17-8483	-	+	+	-	-	-	small	M	round vesicular nuclei	-	-	moderate amount of cytoplasm	5/10hpf	not seen	17-525	neg	neg	neg	3+	C	D	
27	PK	50	F	4.8.17	296855	Pain abdomen ,hypertension	Not available	Adrenal	17-7669	-	+	-	-	-	-	small	M	round vesicular nuclei	-	-	moderate amount of cytoplasm	2/10hpf	not seen	17-522	3+	C	D	3+	C	D	
28	BK	42	F	23.8.17	322394	loss of weight,Pain abdomen	Not available	Appendix	17-8981	-	+	-	-	-	-	small	M	round nuclei with stippled chromatin	-	-	moderate amount of cytoplasm	not seen	not seen	17-551	3+	C	D	3+	C	D	
29	MJ	47	M	28.8.17	325028	loss of weight,Pain abdomen	Not available	Small intestine	17-9144	-	+	-	-	-	-	small	M	round vesicular nuclei	-	-	moderate amount of cytoplasm	2/10hpf	not seen	17-549	3+	C	D	3+	C	D	
30	KK	54	F	5.9.17	329199	Lump in breast ,discharge from nipple	Not available	Breast	17-9642	-	+	-	-	-	islands	small	M	round vesicular nuclei	-	-	moderate amount of cytoplasm	2/10hpf	not seen	17-571	3+	C	D	3+	C	D	
31	MK	47	F	6.9.17	305512	cough ,breathlessness	Not available	Lung	17-9572	-	+	-	-	+	-	large	P	round vesicular nuclei	-	+	moderate amount of cytoplasm	16/10hpf	not seen	17-567	3+	C	D	3+	C	D	
32	VK	66	F	15.9.17	333933	loss of weight,Pain abdomen	Not available	Small intestine	17-9888	-	+	-	+	-	cribiform	small	M	round nuclei with stippled chromatin	-	-	moderate amount of cytoplasm	8/10hpf	not seen	17-577	neg	neg	neg	3+	C	D	
33	DS	23	M	18.10.17	361317	loss of weight	Not available	Liver	17-11838	-	+	-	-	-	-	large	P	round vesicular nuclei	-	-	moderate amount of cytoplasm	30/10hpf	not seen	17-662	3+	C	D	3+	C	D	
34	NS	82	M	30.10.17	350950	Breathlessness	Not available	Lung	17-12016	-	+	-	-	+	-	large	P	round vesicular nuclei	-	+	moderate amount of cytoplasm	22/10hpf	extensive	17-680	3+	C	D	1+	C	F	
35	VD	65	F	5.11.17	390581	Chest pain	Not available	Lung	17-13937	-	-	-	-	+	-	large	P	round vesicular nuclei	-	+	moderate amount of cytoplasm	25/10hpf	focally present	17-774	3+	C	D	2+	C	F	
36	TR	68	M	11.11.17	180333	Breathlessness	Not available	Lung	17-13649	-	-	-	-	+	-	large	P	round vesicular nuclei	-	+	moderate amount of cytoplasm	22/10hpf	extensive	17-786	2+	C	F	2+	C	F	
37	PK	53	F	19.12.17	401429	Loss of appetite ,loss of weight	Not available	Liver	17-14244	-	+	+	-	-	cribiform	small	M	round nuclei with stippled chromatin	-	-	moderate amount of cytoplasm	2/10hpf	not seen	17-795	2+	C	D	not done			
38	SD	45	F	12.1.2018	407896	pain abdomen	not available	Liver	17-14514	-	+	-	-	+	-	small	P	round hyperchromatic nuclei	+	-	scant amount of cytoplasm	25/10hpf	not seen	18-6	neg	neg	neg	3+	C	D	
39	HS	67	M	12.1.2018	411752	cough,breathlessness	not available	Lymph Node	17-14765	-	-	-	-	+	-	large	P	round vesicular nuclei	-	+	scant amount of cytoplasm	15/10hpf	not seen	18-16	3+	C	F	neg	neg	neg	
40	TK	74	F	19.2.2018	426164	backache	not available	Pancreas	18-1388	-	+	-	-	-	-	large	P	round vesicular nuclei	-	+	moderate amount of cytoplasm	22/10hpf	not seen	18-80	3+	C	D	3+	C	D	
41	BK	43	F	16.3.2018	455247	bleeding,loss of weight	not available	Large intestine	18-2674	-	-	-	-	+	cords	small	P	round hyperchromatic nuclei	+	-	scant amount of cytoplasm	25/10hpf	not seen	18-125	3+	C	F	3+	C	F	
42	BK	57	F	16.3.2018	455958	Pain abdomen	not available	Liver	18-2682	-	+	-	-	-	islands	small	M	round vesicular nuclei	-	-	moderate amount of cytoplasm	1/10hpf	not seen	18-122	3+	C	F	3+	C	D	
43	BS	71	M	14.4.2018	464523	Pain abdomen ,weight loss	CT -a hyodense mass seen in right quadrant	Liver	18-3962	-	+	-	-	+	-	large	P	round vesicular nuclei	-	-	moderate amount of cytoplasm	22/10hpf	not seen	18-172	3+	C	D	3+	C	D	

S.NO	Name	Age	Sex	Date	MRD-NO	Chief complaints	Radiology -CT/MRI/US	Location	Histo -no	Architecture						Cell Size	Pleomorphic	Nuclear Features	Spindling	Nucleoli	Cytoplasm	Mitosis	Necrosis	IHC -NO	Chromogranin			Synaptophysin			
										Glands	Nests	Trabecular	Zell ballen	Sheets	Others									Intensity	location	Distrubtion	Intensity	location	Distrubtion	Intensity
44	KL	53	M	14.4.2018	429019	fever ,loss of weight	not available	Lymph node	18-3612	-	-	-	-	+	-	large	P	round vesicular nuclei	-	+	moderate amount of cytoplasm	25/10hpf	not seen	18-161	3+	C	D	3+	C	D	
45	PW	70	F	20.4.2018	467499	Loss of weight ,loss of appetite	not available	Stomach	18-3836	-	+	-	-	-	-	small	M	round nuclei with stippled chromatin	-	-	moderate amount of cytoplasm	1/10hpf	not seen	18-181	3+	C	D	3+	C	D	2+
46	AR	35	F	24.4.2018	479177	Loss of weight ,loss of appetite	not available	Lymph Node	18-4604	-	+	-	-	-	-	small	P	round hyperchromatic nuclei	+	-	scant amount of cytoplasm	22/10hpf	not seen	18-195	3+	C	D	3+	C	D	
47	NP	54	F	30.4.2018	478770	Pain abdomen ,weight loss	not available	Stomach	18-4371	-	+	-	-	-	-	small	M	round nuclei with stippled chromatin	-	-	moderate amount of cytoplasm	1/10hpf	not seen	18-201	3+	C	D	3+	C	D	
48	LS	51	M	30.4.2018	59065	fever,loss of appetite	Mass in left lobe of lung	Lymph node	18-4375	-	-	+	+	-	-	small	M	round vesicular nuclei	-	-	moderate amount of cytoplasm	4/10hpf	not seen	18-198	3+	C	D	3+	C	D	3+
49	JS	38	M	18.05.2018	491231	Pain abdomen ,weight loss	not available	Liver	18-5401	-	+	+	-	-	-	small	P	round hyperchromatic nuclei	+	-	scant amount of cytoplasm	10/10hpf	not seen	18-235	3+	C	D	3+	C	D	
50	AS	55	M	24.05.2018	488977	loss of weight,loss of appetite	not available	Large intestine	18-5240	-	+	+	-	-	-	small	M	round nuclei with stippled chromatin	-	-	moderate amount of cytoplasm	1/10hpf	not seen	18-251	3+	C	F	3+	C	D	neg
51	JK	59	F	4.6.2018	473300	Backhache	Not available	Lymph Node	18-6535	-	+	-	-	-	-	small	P	round hyperchromatic nuclei	+	-	scant amount of cytoplasm	22/10hpf	not seen	18-234	2+	C	F	neg	neg		neg
52	HK	57	F	5.06.2018	504079	diarrhoea,loss of weight	not available	Large intestine	18-6095	-	-	-	-	+	-	small	P	round hyperchromatic nuclei	+	-	scant amount of cytoplasm	26/10hpf	extensive	18-276	3+	C	F	3+	C	D	
53	RK	55	F	9.07.2018	489604	cough,breathlessness	Solid lesion in left lobe of lung	Lung	18-7662	-	+	-	-	+	-	small	M	round hyperchromatic nuclei	-	-	moderate amount of cytoplasm	2/10hpf	not seen	18-328	3+	C	D	3+	C	D	
54	RS	78	M	13.07.2018	528707	Pain abdomen ,weight loss	not available	Lymph node	18-8006	-	+	-	-	+	-	small	P	round hyperchromatic nuclei	+	-	scant amount of cytoplasm	12/10hpf	extensive	18-336	3+	C	D	3+	C	D	
55	BS	44	M	25.7.2018	531516	Loss of weigh ,loss of appetite	not available	Stomach	18-8120	-	+	-	-	-	-	small	M	round nuclei with stippled chromatin	-	-	moderate amount of cytoplasm	2/10hpf	not seen	18-350	3+	C	D	3+	C	D	
56	NLS	50	M	1.8.2018	500892	Backache	not available	Pancreas	18-9137	-	+	-	+	-	-	large	P	round hyperchromatic nuclei	-	-	moderate amount of cytoplasm	22/10hpf	not seen	18-376	3+	C	F	3+	C	D	
57	NK	63	F	7.9.2018	565558	Pain abdomen	not available	Pancreas	18-10497	-	-	+	-	-	cords	small	M	round nuclei with stippled chromatin	-	-	moderate amount of cytoplasm	1/10hpf	not seen	18-456	3+	C	D	3+	C	D	
58	HR	60	M	25.10.2018	579083	Vomiting ,weight loss	Lesion seen in right lobe of lung	Liver	18-11911	-	+	-	-	-	islands	small	P	round hyperchromatic nuclei	+	-	scant amount of cytoplasm	10/10 hpf	focally present	18-553	3+	C	D	3+	C	D	neg
59	SK	75	F	27.10.2018	599484	Pain abdomen ,weight loss	not available	Ampulla	18-12776	-	+	-	-	+	-	large	P	round vesicular nuclei	-	+	moderate amount of cytoplasm	22/10hpf	not seen	18-569	neg	neg	neg	3+	C	D	
60	MS	28	M	29.10.2018	601546	Pain abdomen ,vomiting	CT -lesion in the perigastric location ? Lymph node	Stomach	18-12950	-	+	+	-	-	-	small	M	round vesicular nuclei	-	-	moderate amount of cytoplasm	2/10hpf	not seen	18-564	3+	C	D	Neg	neg	neg	
61	MH	45	M	30.10.2018	635058	vomiting ,fever	not available	Liver	18-12987	-	+	-	+	-	-	large	P	round vesicular nuclei	-	+	moderate amount of cytoplasm	22/10hpf	not seen	19-01	2+	C	F	3+	C	D	2+
62	SA	67	F	3.12.2018	620653	loss of weight	not available	Lymph node	18-14025	-	+	-	+	-	-	large	P	round vesicular nuclei	-	+	moderate amount of cytoplasm	15/10hpf	focally present	18-140	neg	neg	neg	2+	C	F	2+
63	RK	56	M	19.12.2018	632440	Pain abdomen	not available	Small intestine	18-14855	-	+	-	-	-	-	large	P	round vesicular nuclei	-	+	moderate amount of cytoplasm	10/10hpf	not seen	18-668	1+	C	F	1+	C	F	neg
64	IDA	82	M	24.12.2018	633143	vomiting ,fever	CT -sol seen in quadrant 4	Liver	18-15437	+	-	-	+	-	-	small	P	round vesicular nuclei	-	-	moderate amount of cytoplasm	not seen	not seen	18-686	3+	C	D	3+	C	D	
65	JS	75	M	15.02.2019	663285	loss of weight ,appetite ,pain abdomen	Not available	Liver	19-1279	+	-	+	-	-	-	large	P	round vesicular nuclei	-	+	moderate amount of cytoplasm	22/10hpf	extensive	19-78	1+	C	F	neg	neg	neg	2+
66	MH	58	M	15.02.2019	657773	Left shoulder pain ,pain abdomen	Not available	Liver	19-1281	-	+	-	-	-	-	large	P	round nuclei with stippled chromatin	-	-	moderate amount of cytoplasm	24/10hpf	extensive	19-56	3+	C	D	neg	neg	neg	neg
67	VK	57	F	12.03.2019	679298	shortness of breath	Not available	Lymph node	19-2268	-	-	-	-	+	-	large	P	round vesicular nuclei	-	+	moderate amount of cytoplasm	22/10hpf	focally present	19-143	3+	C	D	3+	C	D	
68	AB	40	M	16.3.2019	56772	loss of weight	Not available	Large intestine	19-2277	-	+	-	-	-	-	small	M	round vesicular nuclei	-	-	moderate amount of cytoplasm	1/10hpf	not seen	19-148	neg	neg	neg	3+	C	D	3+
69	DNS	57	M	21.3.2019	665996	pain abdomen	Not available	Adrenal	19-2362	-	-	-	+	-	-	small	M	round vesicular nuclei	-	-	abundant amount of cytoplasm	4/10hpf	not seen	19-163	3+	C	D	3+	C	D	neg
70	NK	64	F	8.04.2019	693285	Loss of weight ,appetite	lesion seen in thyroid	Liver	19-3088	-	+	-	-	+	-	small	P	round hyperchromatic nuclei	+	-	scant amount of cytoplasm	22/10hpf	not seen	19-198	neg	neg	neg	3+	C	D	3+
71	NB	70	F	25.04.2019	703230	Lump right side breast ,loss of appetite	CT -Well defined mass lesion in right breast	Breast	19-4059	-	+	-	-	-	islands	small	M	round nuclei with stippled chromatin	-	-	moderate amount of cytoplasm	2/10hpf	not seen	19-250	3+	C	D	3+	C	D	
72	NM	22	F	2.5.2019	713802	backache,loss of weight	Not available	Pancreas	19-4596	+	-	+	+	-	gyriform	small	M	round nuclei with stippled chromatin	-	-	moderate amount of cyoplasm	3/10hpf	not seen	19-284	2+	C	F	3+	C	F	
73	SS	65	M	4.5.2019	719329	Cough ,shortness of breath	Not available	Lymph node	19-4979	-	+	-	-	+	-	small	P	round hyperchromatic nuclei	+	-	scant amount of cytoplasm	25/10hpf	focally present	19-276	neg	neg	neg	not done			
74	BK	60	M	4.5.2019	705877	Loss of weight ,appetite	lesion in right retroperitoneum	toneal mass	19-4986	-	+	-	+	+	-	small	P	round vesicular nuclei	-	-	moderate amount of cytoplasm	2/10hpf	not seen	19-288	neg	neg	neg	3+	C	D	3+
75	AS	72	M	5.5.2019	725462	Loss of weight ,appetite	MRI-Multiple Lesions in both lobes of liver	Liver	19-5784	-	+	+	-	-	-	small	M	round nuclei with stippled chromatin	-	-	moderate amount of cytoplasm	1/10hpf	not seen	19-319	neg	neg	neg	3+	C	D	3+
76	MK	65	M	6.5.2019	730088	Abdominal discomfort	Not available	Ampulla	19-5875	-	+	-	+	-	-	small	M	round nuclei with stippled chromatin	-	-	abundant amount of cytoplasm	1/10hpf	not seen	19-364	3+	C	D	3+	C	D	
77	TKD	53	F	3.6.2019	738821	Dysnea	Lesion seen in right lobe of lung	Lung	19-6493	-	-	-	-	+	-	large	P	round vesicular nuclei	-	+	abundant amount of cyoplasm	16/10hpf	extensive	19-366	3+	C	D	neg	neg	neg	
78	SS	63	M	13.6.2019	723127	pain abdomen	Not available	Liver	19-6786	-	+	+	-	-	-	small	M	round vesicular nuclei	-	-	moderate amount of cytoplasm	3/10hpf	not seen	19-396	3+	C	D	3+	C	D	3+
79	NK	58	F	15.6.2019	736951	loss of weight ,dysnea	Mass in left lobe of lung	Lymph node	19-6889	-	-	-	-	+	-	small	M	round vesicular nuclei	-	-	moderate amount of cytoplasm	3/10hpf	not seen	19-379	3+	C	D	3+	C	D	
80	SS	73	M	19.6.2019	738766	pain abdomen	Not available	Liver	19-6971	-	+	-	-	+	-	large	P	round vesicular nuclei	-	+	moderate amount of cyoplasm	22/10hpf	extensive	19-408	3+	C	D	3+	C	D	
81	SB	56	M	21.06.2019	738949	cough,loss of weight	Not available	Lymph Node	19-6982	-	+	-	-	+	-	large	P	round vesicular nuclei	-	+	moderate amount of cyoplasm	12/10hpf	focally present	19-401	1+	C	D	2+	C	D	
82	SKM	46	M	1.07.2019	757334	pain abdomen	Not available	Liver	19-7948	-	+	-	+	-	-	small	M	round nuclei with stippled chromatin	-	-	moderate amount of cyoplasm	3/10hpf	not seen	19-449	2+	C	D	2+	C	D	
83	SK	58	F	1.07.2019	744523	Loss of weight ,appetite	Arterially enchancing lesion in left subhepatic region -exophytic pancreatic lesion	Pancreas	19-7994	-	+	-	+	-	-	small	M	round vesicular nuclei	-	-	moderate amount of cyoplasm	1/10hpf	not seen	19-419	3+	C	D	2+	C	F	2+
84	BS	75	M	3.07.2019	591617	Pain abdomen	wall thickening involving 1 st part of Small	Large intestine	19-8024	-	+	+	-	-	-	small	M	round nuclei with stippled chromatin	-	-	moderate amount of cyoplasm	1/10hpf	not seen	19-465	3+	C	D	2+	C	D	2+

S.NO	Name	Age	Sex	Date	MRD-NO	Chief complaints	Radiology -CT/MRI/US	Location	Histo -no	Architecture						Cell Size	Pleomorphic	Nuclear Features	Spindling	Nucleoli	Cytoplasm	Mitosis	Necrosis	IHC -NO	Chromogranin			Synaptophysin			
										Glands	Nests	Trabecular	Zell ballen	Sheets	Others									Intensity	location	Distrubtion	Intensity	location	Distrubtion	Intensity
85	PS	66	M	5.07.2019	756998	Pain abdomen,Loss of appetite	Not available	Lymph node	19-8070	-	+	-	-	+	-	small	M	round nuclei with stippled chromatin	-	-	moderate amount of cyoplasm	2/10hpf	not seen	19-464	3+	C	D	3+	C	D	2+
86	PJ	18	M	15.07.2019	761735	fever	Solid lesion in right middle lobe of lung	Lymph node	19-8275	+	+	-	-	-	cords	large	P	round vesicular nuclei	-	+	moderate amount of cytoplasm	22/10hpf	extensive	14-492	3+	C	D	3+	C	D	1+
87	KS	65	M	16.07.2019	763093	Loss of weight ,appetite	Not available	Lymph node	19-8356	-	-	-	-	+	-	small	P	round hyperchromatic nuclei	+	-	scant amount of cytoplasm	24/10hpf	focally present	19-497	not done			3+	C	D	
88	HRC	67	M	21.7.2019	753737	breathlessness	Not available	Lung	19-8456	-	-	-	-	+	-	large	P	round vesicular nuclei	-	+	moderate amount of cytoplasm	12/10hpf	extensive	19-459	2+	C	D	3+	C	D	
89	SK	18	F	5.8.2019	770514	loss of weight,appetite	Not available	Pancreas	19-9111	-	+	+	-	-	gyriform	small	M	round vesicular nuclei	-	-	moderate amount of cytoplasm	1/10hpf	not seen	19-554	3+	C	F	3+	C	D	
90	Kk	55	F	15.8.2019	735465	Cough ,shortness of breath	Mass seen in the upper lobe of right lung	Lung	19-9493	-	+	-	-	+	-	small	P	round hyperchromatic nuclei	-	-	moderate amount of cytoplasm	6/10hpf	not seen	19-480	not done			3+	C	D	
91	PD	60	M	19.08.2019	779958	pain abdomen	Mass seen in right lung	Liver	19-9915	-	+	+	-	-	-	small	M	round vesicular nuclei	-	-	abundant amount of cytoplasm	4/10hpf	focally present	19-606	3+	C	D	3+	C	D	
92	MK	48	M	20.08.2019	279833	melena,loss of appetite	Not available	Large intestine	19-10143	-	+	-	+	-	-	small	M	round nuclei with stippled chromatin	-	-	moderate amount of cytoplasm	2/10hpf	not seen	19-605	neg	neg	neg	3+	C	D	
93	KS	72	M	30.8.2019	788129	jaundice,pain abdomen	Mass seen in the right lung	Liver	19-10203	-	+	-	-	-	-	small	P	round hyperchromatic nuclei	+	-	scant amount of cytoplasm	22/10hpf	focally present	19-612	3+	C	D	neg	neg	neg	3+
94	SK	64	M	31.08.2019	792283	fever,pain abdomen	Not available	Liver	19-10511	-	+	-	-	-	-	small	P	round hyperchromatic nuclei	+	-	scant amount of cytoplasm	25/10hpf	not seen	19-637	neg	neg	neg	neg	neg	neg	2+
95	BK	56	F	10.09.2019	801035	pain abdomen,loss of appetite,weight	Not available	Liver	19-10835	-	-	-	-	+	-	large	P	plasmacytoid cells with round hyperchromatic nuclei	-	-	moderate amount of cytoplasm	22/10hpf	not seen	19-667	neg	neg	neg	3+	C	D	3+
96	SK	67	M	10.09.2019	755210	jaundice,pain abdomen	Mass in left lobe of lung	Liver	19-107322	-	+	-	-	+	-	large	P	round vesicular nuclei	-	+	moderate amount of cytoplasm	12/10hpf	not seen	19-666	3+	C	D	3+	C	D	
97	VK	67	M	20-9.2019	786650	Loss of weight ,appetite	Not available	Pancreas	19-10998	+	+	-	-	-	-	small	M	round vesicular nuclei	-	-	abundant amount of cytoplasm	12/10hpf	focally present	19-691	neg	neg	neg	3+	C	D	
98	DK	70	M	4.10.2019	812951	dyspepsia	Not available	Stomach	19-11895	-	+	-	-	+	-	large	P	round vesicular nuclei	-	+	moderate amount of cytoplasm	22/10hpf	focally present	19-734,705	neg	neg	neg	neg	neg	neg	3+
99	KS	69	M	1.11.2019	791495	Hematuria	Not available	Prostrate	19-12309	-	-	-	-	+	cribiform	small	P	round hyperchromatic nuclei	+	-	scant amount of cytoplasm	24/10hpf	not seen	19-762	3+	C	D	3+	C	F	
100	AK	70	M	5.11.2019	849394	hypertension ,dyspepsia	Not available	Small intestine	19-12344	+	+	-	+	-	cribiform	small	M	round nuclei with stippled chromatin	-	-	moderate amount of cytoplasm	1/10hpf	not seen	19-911	3+	C	D	3+	C	D	
101	JK	55	F	15.11.2019	842625	loss of appetite ,weight	CT-arterially enchancing lesion in left lobe of liver	Liver	19-13387	+	+	-	-	-	-	small	M	round nuclei with stippled chromatin	-	-	abundant amount of cytoplasm	2/10hpf	not seen	19-858	3+	C	D	3+	C	D	
102	DD	55	F	3.12.2019	844351	Cough ,shortness of breath	Not available	Lung	19-13503	-	+	-	-	+	-	small	P	round hyperchromatic nuclei	+	-	moderate amount of cytoplasm	12/10hpf	extensive	19-897	3+	C	D	3+	C	D	3+
103	ID	80	F	6.12.2019	853509	loss of weight ,backache	Mass in right lobe of lung	Lymph node	19-14210	-	+	-	-	-	cords	small	M	round nuclei with stippled chromatin	-	-	moderate amount of cytoplasm	1/10hpf	not seen	19-912	3+	C	D	3+	C	D	
104	MS	70	M	2.1.20	876486	shoulder pain,epigastric pain	Ct -mediastinal mass in tracheosophageal groove	Esophagus	20-368	-	+	-	-	-	-	small	P	round hyperchromatic nuclei	+	-	scant amount of cytoplasm	22/10hpf	focally present	20-25	neg	neg	neg	neg	neg	neg	3+
105	RKU	70	M	10.1.20	866169	PA,Vomiting	Stricture of one of jejunal loop	Small intestine	20-410	-	+	-	-	-	-	small	M	round nuclei with stippled chromatin	-	-	abundant amount of cytoplasm	1/10hpf	not seen	20-49	3+	C	D	3+	C	D	1+
106	JK	58	M	15.1.20	897890	Pain abdomen	Not available	Lymph node	20-1158	-	-	+	-	-	gyriform	small	M	round vesicular nuclei	-	-	abundant amount of cytoplasm	22/10hpf	not seen	20-72	neg	neg	neg	3+	C	D	
107	AS	45	M	5.2.20	889664	Loss of appetite,loss of weight	Not available	Liver	20-1165	-	+	+	-	-	-	small	M	round nuclei with stipled chromatin	-	-	moderate amount of cytoplasm	1/10hpf	not seen	20-79	2+	C	F	3+	C	D	
108	SC	57	M	12.2.20	808964	pain abdomen ,fever	CT -soft tissue lesion in mesentry s/o carcinoid tumor	Liver	20-1388	+	+	-	-	-	-	small	M	round nuclei with stippled chromatin	-	-	moderate amount of cytoplasm	1/10hpf	not seen	20-97	3+	C	D	3+	C	D	
109	RK	42	M	12.2.20	885974	diarrhoea	Not available	Small intestine	20-1874	-	+	-	-	-	-	small	M	round nuclei with stippled chromatin	-	-	moderate amount of cytoplasm	1/10hpf	not seen	20-99	3+	C	F	3+	C	D	
110	AK	41	M	19.2.20	605277	Loss of appetite,diarrhoea,weakness	CT -Hepatic flexure thickening -s/o-lymphoma,adenocarcinoma	Large intestine	20-1899	nests	-	-	-	-	-	small	M	round hyperchromatic nuclei	-	-	scant amount of cytoplasm	1/10hpf	not seen	20-104	3+	C	F	3+	C	F	
111	BS	58	M	2.3.20	903352	Cough ,breathlessness	Not available	Lung	20-2072	-	+	-	-	-	-	small	P	round hyperchromatic nuclei	+	-	scant amount of cytoplasm	12/10hpf	not seen	20-156	2+	C	D	3+	C	D	
112	SK	67	F	19.3.20	909005	pain abdomen ,loss of weight	Not available	Pancreas	20-2484	-	+	-	+	-	-	small	M	round nuclei with stippled chromatin	-	-	moderate amount of cytoplasm	2/10 hpf	not seen	20-203	3+	C	D	3+	C	D	
113	MS	69	M	25.3.20	838772	Cough ,breathlessness	Not available	Lymph Node	20-2257	-	+	-	-	-	-	small	M	round vesicular nuclei	-	-	moderate amount of cytoplasm	8/10hpf	focally present	20-198	2+	C	D	2+	C	D	
114	MG	72	M	2.4.20	346755	Loss of appetite,loss of weight	Not available	Liver	20-3278	-	+	-	-	+	-	small	P	round hyperchromatic nuclei	+	-	scant amount of cytoplasm	22/10hpf	extensive	20-250	neg	neg	neg	3+	C	D	2+
115	HR	56	M	15.4.20	908593	Pain abdomen	Not available	Liver	20-3361	-	+	-	-	+	-	small	P	round hyperchromatic nuclei	+	-	scant amount of cytoplasm	32/10hpf	not seen	20-219	3+	C	D	3+	C	D	
116	CS	60	M	1.5.20	919948	fever	Not available	Lymph node	20-3384	-	+	-	-	-	cords	small	M	round vesicular nuclei	-	-	moderate amount of cytoplasm	1/10hpf	not seen	20-296	2+	C	F	3+	C	D	
117	TD	45	M	14.5.20	921333	Pain abdomen	Not available	Lymph node	20-3411	-	+	-	-	-	-	large	P	round vesicular nuclei	-	+	moderate amount of cytoplasm	15/10hpf	extensive	20-279	neg	neg	neg	3+	C	D	
118	ISR	75	M	14.5.20	924843	Generalised weakness,decreased appetite	Not available	Liver	20-3675	-	+	-	-	+	-	large	P	round vesicular nuclei	-	+	moderate amount of cytoplasm	34/10hpf	not seen	20-326	3+	C	D	3+	C	D	
119	HC	55	M	16.5.20	924834	Loss of appetite,loss of weight	Not available	Lymph node	20-3680	-	+	-	-	-	-	small	M	round vesicular nuclei	-	-	abundant amount of cytoplasm	2/10hpf	focally present	20-334	3+	C	D	3+	C	D	
120	ND	55	F	16.5.20	743519	generalised weakness	Not available	Liver	20-3690	-	+	-	-	+	-	small	P	round hyperchromatic nuclei	+	-	scant amount of cytoplasm	not seen	extensive	20-337	2+	C	D	3+	C	D	
121	AS	57	M	18.5.20	919295	generalised weakness,fever	Not available	Liver	20-3811	-	+	-	-	+	-	small	P	round hyperchromatic nuclei	+	-	scant amount of cytoplasm	28/10 hpf	focally present	20-246	3+	C	D	3+	C	D	
122	PS	59	M	12.6.20	925087	neck swelling ,fever	Not available	Lymph node	20-4095	nests						large	P	round vesicular nuclei	-	+	moderate amount of cytoplasm	22/10 hpf	focally present	20-412	3+	C	D	neg	neg	neg	neg
123	RR	47	F	16.6.20	924906	fever	Not available	Liver	20-4631	-	+	-	-	-	-	small	P	round vesicular nuclei	-	-	moderate amount of cytoplasm	26/10hpf	not seen	20-424	3+	C	D	neg	neg	neg	3+

S.NO	Name	Age	NSE		CD56			Others				Ki-67	Diagnosis	Final Diagnosis
			location	Distrubtion	Intensity	location	Distrubtion	TTF-1	CEA	CDX2	OTHERS			
1	BK	58	Not done			Not done		-	pos	-	not done	1%	Neuroendocrine tumor grade 1	Neuroendocrine tumor grade 1
2	NR	58	Not done			Not done		pos	-	-	not done	60%	Metastatic deposits from Small cell carcinoma -lung	Metastatic deposits from small cell carcinoma -lung
3	JK	58	Not done			Not done		-	-	-	not done	50%	Small cell carcinoma	Small cell carcinoma
4	SS	45	Not done			Not done		-	-	pos	not done	3%	Neuroendocrine tumor grade 2	Neuroendocrine tumor grade 2
5	SS	44	Not done			Not done		-	-	-	not done	2%	Neuroendocrine tumor grade 1	Neuroendocrine tumor grade 1
6	PL	66	Not done			Not done		-	-	-	not done	70%	Large cell neuroendocrine carcinoma	Large cell neuroendocrine carcinoma
7	BR	72	Not done			Not done		-	-	-	not done	3%	Neuroendocrine tumor grade 2	Neuroendocrine tumor grade 2
8	DS	60	C	D		Not done		pos	-	-	not done	50%	Metastatic deposits from large cell neuroendocrine carcinoma -lung	Metastatic deposits from large cell neuroendocrine carcinoma -lung
9	HS	68	Not done			Not done		-	-	pos	not done	2%	Metastatic deposits from neuroendocrine tumor grade 1- GI in origin	Metastatic deposits from neuroendocrine tumor grade 1- GI in origin
10	AD	48	C	D		Not done		-	-	pos	not done	1%	Neuroendocrine tumor grade 1	Neuroendocrine tumor grade 1
11	ICJ	70	Not done			Not done		-	-	-	not done	50%	Large cell neuroendocrine carcinoma	Large cell neuroendocrine carcinoma
12	GL	64	Not done			Not done		-	-	pos	not done	1%	Neuroendocrine tumor grade 1	Neuroendocrine tumor grade 1
13	KM	50	Not done			Not done		-	-	pos	not done	1%	Neuroendocrine tumor -grade 1	Neuroendocrine tumor -grade 1
14	DK	58	Not done			Not done		-	-	pos	not done	1%	Metastatic deposits from neuroendocrine tumor - grade 1 -GI in origin	Metastatic deposits from neuroendocrine tumor -grade 1 -GI in origin
15	KK	30	not done			not done		-	-	-	S-100-pos	20%	Neuroendocrine tumor -pheochromocytoma	Neuroendocrine tumor -pheochromocytoma
16	RK	60	not done			not done		-	-	-	not done	80%	Large cell neuroendocrine carcinoma	Large cell neuroendocrine carcinoma
17	SW	24	not done			not done		-	-	-	ER -7/8,PR-6/8,HER-neg	10%	Invasive duct carcinoma with neuroendocrine differentation	Invasive duct carcinoma with neuroendocrine differentation
18	MS	45	not done			not done		-	-	-	not done	4%	Metastatic deposits from atypical carcinoid -lung	Metastatic deposits from atypical carcinoid -lung
19	JS	65	not done			not done		pos	-	-	not done	80%	Small cell carcinoma	Small cell carcinoma
20	GK	62	not done			not done		-	-	-	not done	1%	Metastatic deposits from typical carcinoid -lung	Metastatic deposits from typical carcinoid -lung
21	GK	55	not done			not done		pos	-	-	not done	20%	Metastatic deposits from atypical carcinoid - lung	Metastatic deposits from atypical carcinoid - lung
22	DK	40	not done			not done		-	-	pos	not done	80%	Metastatic deposits of large cell neuroendocrine carcinoma -GI in origin	Metastatic deposits of large cell neuroendocrine carcinoma -GI in origin
23	KS	34	not done			not done		-	-	-	not done	2%	Neuroendocrine tumor -grade 1	Neuroendocrine tumor -grade 1
24	AK	60	not done			not done		-	-	-	S-100-pos	20%	Neuroendocrine tumor -pheochromocytoma	Neuroendocrine tumor -pheochromocytoma
25	KD	70	not done			not done		-	-	-	not done	1%	Neuroendocrine tumor -grade 1	Neuroendocrine tumor -grade 1
26	SK	59	not done			not done		-	-	-	ER -8/8,PR-2/8,HER-2+	2%	Invasive duct carcinoma with neuroendocrine differentation	Invasive duct carcinoma with neuroendocrine differentation
27	PK	50	not done			not done		-	-	-	S-100-pos	2%	Neuroendocrine tumor -pheochromocytoma	Neuroendocrine tumor -pheochromocytoma
28	BK	42	not done			not done		-	-	-	not done	1%	Neuroendocrine tumor -grade 1	Neuroendocrine tumor -grade 1
29	MJ	47	not done			not done		-	-	pos	not done	1%	Neuroendocrine tumor -grade 1	Neuroendocrine tumor -grade 1
30	KK	54	not done			not done		-	-	-	ER-8/8,PR-4/8,HER-neg	12%	Invasive duct carcinoma with neuroendocrine differentation	Invasive duct carcinoma with neuroendocrine differentation
31	MK	47	not done			not done		-	-	-	not done	70%	Large cell neuroendocrine carcinoma	Large cell neuroendocrine carcinoma
32	VK	66	not done			not done		-	-	-	not done	4%	Neuroendocrine tumor -grade 2	Neuroendocrine tumor -grade 2
33	DS	23	not done			not done		-	-	pos	not done	25%	Metastatic deposits from large cell neuroendocrine carcinoma -GI in origin	Metastatic deposits from neuroendocrine tumor grade 3-GI in origin
34	NS	82	not done			not done		-	-	-	not done	60%	large cell neuroendocrine carcinoma	Large cell neuroendocrine carcinoma
35	VD	65	not done			not done		-	-	-	not done	75%	large cell neuroendocrine carcinoma	Large cell neuroendocrine carcinoma
36	TR	68	not done			not done		-	-	-	not done	60%	large cell neuroendocrine carcinoma	Large cell neuroendocrine carcinoma
37	PK	53	not done			not done		-	-	-	not done	1%	Neuroendocrine tumor -grade 1	Neuroendocrine tumor -grade 1
38	SD	45	not done			not done		-	-	pos	not done	70%	Metastatic deposits from small cell neuroendocrine carcinoma -GI in origin	Metastatic deposits from small cell neuroendocrine carcinoma -GI in origin
39	HS	67	not done			not done		-	-	pos	not done	90%	Metastatic deposits from large cell neuroendocrine carcinoma - GI in origin	Metastatic deposits from large cell neuroendocrine carcinoma - GI in origin
40	TK	74	not done			not done		-	pos	-	not done	50%	Large cell neuroendocrine carcinoma	Neuroendocrine tumor -grade 3
41	BK	43	not done			not done		-	-	-	not done	70%	Small cell neuroendocrine carcinoma	Small cell neuroendocrine carcinoma
42	BK	57	not done			not done		-	-	pos	not done	2%	Metastatic deposits from neuroendocrine tumor grade 1-GI in origin	Metastatic deposits from neuroendocrine tumor grade 1-GI in origin
43	BS	71	not done			not done		-	-	pos	not done	50%	Metastatic deposits from large cell neuroendocrine carcinoma -GI in origin	Metastatic deposits from large cell neuroendocrine carcinoma -GI in origin

S.NO	Name	Age	NSE		CD56			Others				Ki-67	Diagnosis	Final Diagnosis
			location	Distrubtion	Intensity	location	Distrubtion	TTF-1	CEA	CDX2	OTHERS			
44	KL	53	not done			not done		-	-	pos	not done	90%	Metastatic deposits from large cell neuroendocrine carcinoma - GI in origin	Metastatic deposits from large cell neuroendocrine carcinoma - GI in origin
45	PW	70	C	F		not done		-	-	-	CK -pos	2%	Neuroendocrine tumor -grade 1	Neuroendocrine tumor -grade 1
46	AR	35	not done			not done		-	-	pos	not done	80%	Metastatic deposits from small cell neuroendocrine carcinoma - GI in origin	Metastatic deposits from small cell neuroendocrine carcinoma - GI in origin
47	NP	54	not done			not done		-	-	pos	not done	2%	Neuroendocrine tumor -grade 1	Neuroendocrine tumor -grade 1
48	LS	51	C	D		not done		-	-	-	not done	2%	Metastatic deposits from typical carcinoid - lung	Metastatic deposits from typical carcinoid - lung
49	JS	38	not done			not done		pos	-	-	not done	55%	Metastatic deposits from small cell carcinoma - lung	Metastatic deposits from small cell carcinoma - lung
50	AS	55	neg	neg		not done		-	-	pos	not done	1%	Neuroendocrine tumor -grade 1	Neuroendocrine tumor -grade 1
51	JK	59	neg	neg		not done		-	pos	-	not done	50%	Metastatic deposits from small cell neuroendocrine carcinoma - Pancreaticobililary in origin	Metastatic deposits from small cell neuroendocrine carcinoma - Pancreaticobiliary in origin
52	HK	57	not done			not done		pos	-	-	not done	80%	Metastatic deposits from small cell carcinoma - lung	Metastatic deposits from small cell carcinoma - lung
53	RK	55	not done			not done		-	-	-	not done	1%	Typical Carcinoid	Typical Carcinoid
54	RS	78	not done			not done		pos	-	-	not done	75%	Metastatic deposits from small cell carcinoma - lung	Metastatic deposits from small cell carcinoma - lung
55	BS	44	not done			not done		-	-	pos	not done	2%	Neuroendocrine tumor -grade 1	Neuroendocrine tumor -grade 1
56	NLS	50	not done			not done		-	pos	-	not done	25%	Large cell neuroendocrine carcinoma	Neuroendocrine tumor -grade 3
57	NK	63	not done			not done		-	pos	-	not done	1%	Neuroendocrine tumor -grade 1	Neuroendocrine tumor -grade 1
58	HR	60	neg	neg	2+	M	D	-	-	-	not done	40%	Metastatic deposits from small cell carcinoma - lung	Metastatic deposits from small cell carcinoma - lung
59	SK	75	not done			not done		-	-	pos	not done	80%	Large cell neuroendocrine carcinoma	Large cell neuroendocrine carcinoma
60	MS	28	not done			not done		-	-	-	not done	2%	Neuroendocrine tumor -grade 1	Neuroendocrine tumor -grade 1
61	MH	45	C	F		not done		-	-	-	SATB2- pos	25%	Metastatic deposits from large cell neuroendocrine carcinoma - GI in origin	Metastatic deposits from neuroendocrine tumor -grade 3 - GI in origin
62	SA	67	C	F	2+	M	D	-	-	pos	not done	50%	Metastatic deposits from large cell neuroendocrine carcinoma - GI in origin	Metastatic deposits from large cell neuroendocrine carcinoma - GI in origin
63	RK	56	neg	neg		not done		-	-	pos	not done	50%	Large cell neuroendocrine carcinoma	Neuroendocrine tumor -grade 3
64	IDA	82	not done			not done		pos	-	-	not done	30%	Metastatic deposits from atypical carcinoid - lung	Metastatic deposits from atypical carcinoid - lung
65	JS	75	C	D		not done		-	-	pos	PSA -neg	60%	Metastatic deposits from large cell neuroendocrine carcinoma -GI in origin	Metastatic deposits from large cell neuroendocrine carcinoma -GI in origin
66	MH	58	neg	neg		not done		-	pos	pos	CK7,CK20-neg	25%	Metastatic deposits from large cell neuroendocrine carcinoma - GI in origin	Metastatic deposits from neuroendocrine tumor -grade 3 - GI in origin
67	VK	57	not done			not done		-	-	pos	not done	60%	Metastatic deposits from large cell neuroendocrine carcinoma - GI in origin	Metastatic deposits from large cell neuroendocrine carcinoma - GI in origin
68	AB	40	C	D		not done		-	-	-	not done	2%	Neuroendocrine tumor -grade 1	Neuroendocrine tumor -grade 1
69	DNS	57	neg	neg	3+	M	D	-	-	-	S-100-pos	3%	Neuroendocrine tumor -pheochromocytoma	Neuroendocrine tumor -pheochromocytoma
70	NK	64	C	D	3+	M	D	pos	-	-	PAX 8 -pos	70%	Metastatic deposits of medullary carcinoma- thyroid	Metastatic deposits of medullary carcinoma- thyroid
71	NB	70	not done		neg	neg	neg	-	-	-	ER-8/8,PR-neg,HER-2/NEU-neg	2%	Neuroendocrine tumor -grade 1	Neuroendocrine tumor -grade 1
72	NM	22	not done			not done		-	pos	-	CK -pos	10%	Neuroendocrine tumor -grade 2	Neuroendocrine tumor -grade 2
73	SS	65	not done		3+	M	D	pos	-	-	not done	95%	Metastatic deposits from small cell carcinoma -lung	Metastatic deposits from small cell carcinoma -lung
74	BK	60	C	D		not done		-	-	-	CK -neg,S-100-pos	70%	Neuroendocrine tumor -paraganglioma	Neuroendocrine tumor -paraganglinoma
75	AS	72	C	D	3+	M	F	-	pos	-	CK7,CK20-neg	2%	Metastatic deposits from neuroendocrine tumor - grade 1-Pancreaticobillary in origin	Metastatic deposits from neuroendocrine tumor -grade 1-Pancreaticobillary in origin
76	MK	65	not done			not done		-	-	pos	not done	2%	Neuroendocrine tumor -grade 1	Neuroendocrine tumor -grade 1
77	TKD	53	not done			not done		-	-	-	not done	65%	Large cell neuroendocrine carcinoma	Large cell neuroendocrine carcinoma
78	SS	63	C	D		not done		-	pos	-	not done	4%	Metastatic deposits from neuroendocrine tumor grade 2 -Pancreaticobillary in origin	Metastatic deposits from neuroendocrine tumor grade 2 -Pancreaticobillary in origin
79	NK	58	not done			not done		pos	-	-	not done	25%	Metastatic deposits from atypical carcinoid - lung	Metastatic deposits from atypical carcinoid - lung
80	SS	73	not done			not done		pos	-	-	PAX 8 - neg	75%	Metastatic deposits from large cell neuroendocrine carcinoma - lung	Metastatic deposits from large cell neuroendocrine carcinoma - lung
81	SB	56	not done			not done		pos	-	-	not done	75%	Metastatic deposits from large cell neuroendocrine carcinoma - lung	Metastatic deposits from large cell neuroendocrine carcinoma - lung
82	SKM	46	not done			not done		-	-	pos	CK7,CK20-neg	15%	Metastatic deposits from neuroendocrine tumor - grade 2 - GI in origin	Metastatic deposits from neuroendocrine tumor -grade 2 - GI in origin
83	SK	58	C	D		not done		-	pos	-	CK7,CK20-neg	2%	Neuroendocrine tumor -grade 1	Neuroendocrine tumor -grade 1
84	BS	75	C	D		not done		-	-	-	not done	1%	Neuroendocrine tumor -grade 1	Neuroendocrine tumor -grade 1

S.NO	Name	Age	NSE		CD56			Others				Ki-67	Diagnosis	Final Diagnosis
			location	Distrubtion	Intensity	location	Distrubtion	TTF-1	CEA	CDX2	OTHERS			
85	PS	66	C	D	1+	M	F	-	-	pos	CK7,CK20-neg	1%	Metastatic deposits from neuroendocrine tumor - grade 1- GI in origin	Metastatic deposits from neuroendocrine tumor -grade 1- GI in origin
86	PJ	18	C	D	not done			pos	-	-	not done	70%	Metastatic deposits from large cell neuroendocrine carcinoma - lung	Metastatic deposits from large cell neuroendocrine carcinoma - lung
87	KS	65	not done		not done			pos	-	-	CK-pos	80%	Metastatic deposits from small cell carcinoma-lung	Metastatic deposits from small cell carcinoma-lung
88	HRC	67	not done		not done			-	-	-	CK-pos	90%	Large cell neuroendocrine carcinoma	Large cell neuroendocrine carcinoma
89	SK	18	not done		neg	neg	neg	-	pos	-	not done	2%	Neuroendocrine tumor -grade 1	Neuroendocrine tumor -grade 1
90	Kk	55	not done		not done			pos	-	-	not done	5%	Atypical carcinoid	Atypical carcinoid
91	PD	60	not done		not done			-	-	-	not done	8%	Metastatic deposits from atypical carcinoid -lung	Metastatic deposits from atypical carcinoid -lung
92	MK	48	not done		not done			-	-	-	not done	2%	Neuroendocrine tumor -grade 1	Neuroendocrine tumor -grade 1
93	KS	72	C	F	neg	neg	neg	-	-	-	not done	70%	Metastatic deposits from small cell carcinoma - lung	Metastatic deposits from small cell carcinoma - lung
94	SK	64	C	F	neg	neg	neg	-	pos	-	not done	70%	Metastatic deposits from small cell neuroendocrine carcinoma - Pancraetocobillary in origin	Metastatic deposits from small cell neuroendocrine carcinoma - Pancraetocobillary in origin
95	BK	56	C	D	not done			-	-	pos	not done	25%	Metastatic deposits from neuroendocrine tumor grade 3 - GI in origin	Metastatic deposits from neuroendocrine tumor grade 3 - GI in origin
96	SK	67	not done		not done			-	-	-	not done	60%	Metastatic deposits from large cell neuroendocrine carcinoma - lung	Metastatic deposits from large cell neuroendocrine carcinoma - lung
97	VK	67	not done		not done			-	pos	-	not done	7%	Neuroendocrine tumor -grade 2	Neuroendocrine tumor -grade 2
98	DK	70	C	D	not done			-	-	-	not done	90%	large cell neuroendocrine carcinoma	large cell neuroendocrine carcinoma
99	KS	69	not done		not done			-	-	-	PSA,AMACR,GATA-3-Positive	40%	Mixed acinar adenocarcinoma and small cell neuroendocrine carcinoma	Mixed acinar adenocarcinoma and small cell neuroendocrine carcinoma
100	AK	70	not done		not done			-	-	-	CK -positive	2%	Neuroendocrine tumor -grade 1	Neuroendocrine tumor -grade 1
101	JK	55	not done		not done			-	-	pos	not done	2%	Metastatic deposits from neuroendocrine tumor grade 1 -GI in origin	Metastatic deposits from neuroendocrine tumor grade 1 -GI in origin
102	DD	55	C	F	neg	neg	neg	-	-	-	not done	50%	Small cell neuroendocrine carcinoma	Small cell neuroendocrine carcinoma
103	ID	80	not done		not done			-	-	-	CK7,CK20-neg	2%	Metastatic deposits from typical carcinoid -lung	Metastatic deposits from typical carcinoid -lung
104	MS	70	C	F	2+	M	F	-	-	-	CK - positive	95%	Small cell carcinoma	Small cell carcinoma
105	RKU	70	C	F	1+	M	F	-	-	pos	not done	2%	Neuroendocrine tumor -grade 1	Neuroendocrine tumor -grade 1
106	JK	58	not done		not done			-	-	pos	not done	22%	Metastatic deposits from large cell neuroendocrine carcinoma- GI in origin	Metastatic deposits from neuroendocrine tumor -grade 3- GI in origin
107	AS	45	not done		not done			-	-	pos	not done	2%	Metastatic deposits from neuroendocrine tumor - grade 1-GI in origin	Metastatic deposits from neuroendocrine tumor -grade 1-GI in origin
108	SC	57	not done		not done			-	-	pos	not done	2%	Metastatic deposits from neuroendocrine tumor - grade 1- GI in origin	Metastatic deposits from neuroendocrine tumor -grade 1- GI in origin
109	RK	42	not done		not done			-	-	-	not done	1%	Neuroendocrine tumor -grade 1	Neuroendocrine tumor -grade 1
110	AK	41	not done		not done			-	-	-	not done	40%	Mixed neuroendocrine- non neuroendocrine neoplasm	Mixed neuroendocrine -non neuroendocrine neoplasm
111	BS	58	not done		not done			pos	-	-	CK -pos	60%	Small cell carcinoma	Small cell carcinoma
112	SK	67	not done		not done			-	-	-	not done	2%	Neuroendocrine tumor -grade 1	Neuroendocrine tumor -grade 1
113	MS	69	not done		not done			pos	-	-	PAX-8-pos	8%	Metastatic deposits from medullary carcinoma-Thyroid	Metastatic deposits from medullary carcinoma -Thyroid
114	MG	72	C	Focal	3+	M	D	pos	-	-	PSA-negative	60%	Metastatic deposits from small cell neuroendocrine Carcinoma -prostrate	Metastatic deposits from small cell neuroendocrine Carcinoma -prostrate
115	HR	56	Not done		not done			pos	-	-	not done	90%	Metastatic deposits from small celll carcinoma-lung	Metastatic deposits from small celll carcinoma-lung
116	CS	60	not done		not done			-	-	pos	not done	1%	Metastatic deposits from neuroendocrine tumor - grade 1-GI in origin	Metastatic deposits from neuroendocrine tumor -grade 1-GI in origin
117	TD	45	not done		not done			pos	-	-	not done	70%	Metastatic deposits from large cell neuroendocrine carcinoma-lung	Metastatic deposits from large cell neuroendocrine carcinoma-lung
118	ISR	75	Not done		not done			-	-	pos	CK7-pos	60%	Metastatic deposits from large cell neuroendocrine carcinoma - GI in origin	Metastatic deposits from large cell neuroendocrine carcinoma - GI in origin
119	HC	55	Not done		not done			-	-	pos	not done	1%	Metastatic deposits from neuroendocrine tumor - grade 1-GI in origin	Metastatic deposits from neuroendocrine tumor -grade 1-GI in origin
120	ND	55	Not done		not done			pos	-	-	not done	70%	Metastatic deposits from small celll carcinoma-lung	Metastatic deposits from small celll carcinoma-lung
121	AS	57	Not done		not done			pos	-	-	not done	60%	Metastatic deposits from small celll carcinoma-lung	Metastatic deposits from small celll carcinoma-lung
122	PS	59	neg	neg	not done			-	-	pos	not done	70%	Metastatic deposits from large cell neuroendocrine carcinoma - GI in origin	Metastatic deposits from large cell neuroendocrine carcinoma - GI in origin
123	RR	47	C	F	not done			-	pos	-	not done	40%	Metastatic deposits from neuroendocrine tumor - grade 3-pancreaticobillary	Metastatic deposits from neuroendocrine tumor -grade 3-pancreaticobillary

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No. BFUHS/2K19p-III/ 10517 Date 5/5/19

The Principal,
DMC, Ludhiana.

Subject: Approval of Plan of Thesis.

This is to inform you that the Faculty of Medical Sciences in its meeting held on 29.05.2019 has approved the plan of thesis of **Harsimran Sandhu**, a student of your college for **MD Pathology** along with the names of Supervisor/Co-Supervisor as under:-

Topic Plan of Thesis
Histopathological spectrum of neuroendocrine tumors

Supervisor
Dr. Harpreet Kaur
Prof.,
Dept. of Pathology,
DMCH, Ludhiana.

Co-Supervisor
Dr. Neena Sood
Prof. & Head,
Dept. of Pathology,
DMCH, Ludhiana.

The student has to submit the four copies of thesis (along with PDF format) , to the Controller of Examinations, BFUHS, Faridkot, at least six months before the commencement of theory examination, if he/she is otherwise eligible to take the examination. He /She must directed to attach a photocopy of this letter with the forwarding letter while submitting thesis to the University along with the letter of approval of University for change of supervisor/Co-Supervisor, change of topic,(if any).

Dr. S P Singh MS(Oph)
Controller of Examinations

Note

1. *If any discrepancy found regarding topic of thesis or Supervisor/Co-Supervisor, inform undersigned within one month after issue of this letter.*
2. *The student may submit the thesis as per the schedule already conveyed i.e for May/June examination 30th Nov of the preceding the year of examination and for Nov/Dec examinations 31st May of the year of examination.*

HISTOPATHOLOGICAL SPECTRUM OF NEUROENDOCRINE TUMORS

THESIS PROPOSAL

FOR APPROVAL OF SUBJECT OF THESIS TO BE SUBMITTED

IN PARTIAL FULFILMENT OF THE REQUIREMENTS

FOR THE DEGREE OF

Doctor of Medicine

in

PATHOLOGY

OF THE

BABA FARID UNIVERSITY OF HEALTH SCIENCES

FARIDKOT

December, 2018

Dr. Harsimran Sandhu

DEPARTMENT OF PATHOLOGY
DAYANAND MEDICAL COLLEGE & HOSPITAL, LUDHIANA
LUDHIANA, PUNJAB-141001

ABSTRACT OF PLAN OF THESIS

Title	Histopathological spectrum of Neuroendocrine Tumors
For the degree of	M.D. Pathology
Name of the candidate	Dr. Harsimran Sandhu
Supervisor	Dr. Harpreet Kaur , Professor, Department of Pathology, DMCH, Ludhiana
Co-supervisor	Dr. Neena Sood, Professor & Head, Department of Pathology, DMCH, Ludhiana
Institution	Dayanand Medical College and Hospital, Ludhiana
University	Baba Farid University of Health Sciences, Faridkot

Introduction: This will be a retrospective and prospective study to be conducted in the department of pathology, Dayanand Medical College & Hospital, Ludhiana. All the cases of IHC proven neuroendocrine tumors will be analysed with respect to microscopy and correlated clinically.

Aim of study: Histopathological spectrum of neuroendocrine tumors.

Materials and methods: All the biopsies as well as resected specimens of neuroendocrine tumors will be included in the study. Tissue will be processed and stained with haematoxylin and eosin. The slides will be subjected to histopathological examination under both low power (10X) and high power (40X).In all cases IHC staining will be done and analysed.

Clinical significance: To obtain reasonable good database for a more accurate assessment and diagnosis of neuroendocrine tumors.

Keywords: neuroendocrine tumors, IHC.

(Signature)	(Signature)	(Signature)	(Signature)
Candidate	Co-supervisor	Supervisor	Principal

CERTIFICATE OF FACILITIES AVAILABLE

This is to certify that the facilities for work on the subject of thesis titled **"HISTOPATHOLOGICAL SPECTRUM OF NEUROENDOCRINE TUMORS"** exist in the Department of Pathology at Dayanand Medical College and Hospital, Ludhiana and will be provided to the candidate. We will see that the data being included in the thesis are genuine and is collected by the candidate herself under our supervision and guidance.

Supervisor

Dr. Harpreet Kaur
Professor
Department of Pathology
DMCH, Ludhiana

Co-Supervisor

Dr. Neena Sood
Professor & Head
Department of Pathology
DMCH, Ludhiana

Dr. Neena Sood
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Department of Pathology
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Place : Ludhiana

Date : 17-12-16

APPROVAL PROFORMA
BY
RESEARCH & ETHICAL COMMITTEE
DAYANAND MEDICAL COLLEGE & HOSPITAL, LUDHIANA

Name of candidate	Dr. Harsimran Sandhu
Department	Pathology
Topic of Thesis	HISTOPATHOLOGICAL SPECTRUM OF NEUROENDOCRINE TUMORS
Likely date of appearing in PG examination	MAY-JUNE 2019
Date of enrollment	MAY 10, 2018
Name of Head of Department	Dr. Neena Sood
1) Supervisor	Dr. Harpreet Kaur
2) Co-Supervisor	Dr. Neena Sood
Signature of Members of Research Committee with Stamp	**Signature of Members of Ethics Committee with Stamp**
1. HOD Pharmacology	1. HOD Physiology
2. HOD Surgery	2. HOD Pathology
3. HOD Pathology	3. HOD Pharmacology
4. HOD Medicine	4. HOD SPM
5. Dean Academics	5. HOD Medicine
6. Vice Principal	6. HOD Surgery
	7. HOD Obst. & Gynae
	8. Legal Expert
	9. Secretary, IEC
	10. Dean Academics
	11. Vice Principal
	12. Principal

Approved: Yes / No

Chairperson
Research Committee

Approved: Yes / No

Chairperson
Ethics Committee

Chairperson
Research Committee

Chairperson
Ethics Committee

BABA FARID UNIVERSITY OF HEALTH SCIENCES, FARIDKOT

PROFORMA FOR REGISTRATION OF SUBJECT FOR DISSERTATION

1	NAME OF THE CANDIDATE AND ADDRESS	Dr. Harsimran Sandhu V.P.O Ladha Munda, Teh-Batala, Distt –Gurdaspur
2	NAME OF THE INSTITUTE	Dayanand Medical College & Hospital, Ludhiana
3	COURSE OF STUDY AND SUBJECT	MD PATHOLOGY
4	DATE OF ADMISSION OF COURSE	10th MAY,2018
5	TITLE OF THE TOPIC	HIATOPATHOLOGICAL SPECTRUM OF NEUROENDOCRINE TUMORS

6. BRIEF RESUME OF THE INTENDED WORK

6.1 NEED FOR STUDY

Neuroendocrine tumors are heterogenous tumors which have a common phenotype and share features of neural and endocrine differentiation such as presence of secretory granules, synaptic like vesicles and production of amine or peptide hormones irresepective of their primary site. The concept of neuroendocrine tumors was developed in early 1900 with description by Obendorfer of carcinoid tumors in 1907.[1-2]

The estimated prevelance of neuroendocrine tumors (NET) is 1 to 2 cases per 100,000 people. Most common site is gastrointestinal tract (GIT).The distribution pattern of NETs in GIT is different between Eastern and Western population. In Western populations most common site in GIT is small intestine (38%) followed by rectum (34%). In Eastern population most common site is rectum (48%) followed by stomach (15%).[3]

There are mainly two different groups of Neuroendocrine Neoplasms:

- Well differentiated /Low proliferating NENs -Neuroendocrine tumors.
- Poorly differentiated NENs/Highly proliferating NENs -Neuroendocrine Carcinoma.[4]

Well differentiated neuroendocrine tumors are arranged in well developed organoid nesting, trabecular, gyriform growth patterns. Tumor cells are small with relatively uniform round to oval nuclei, inconspicuous nucleoli and salt and pepper chromatin. Neuroendocrine carcinomas demonstrate solid proliferation of tumor cells with irregular nuclei and high mitotic figures.[5]

Elevated levels of serum chromogranim A act as a sensitive marker for detection of neuroendocrine neoplasm. It may indicate neuroendocrine

differentiation in tumor.[6] Markers such as Chromogranin A, Serum Neuron Specific Enolase, subunit of glycoprotein hormones have been used for screening purposes.[7]

Neuroendocrine neoplasms share expression of markers such as Synaptophysin and Chromogranin A. Morphology may be sufficient to recognize neuroendocrine tumors, but IHC is always required for confirming the diagnosis. Thus a correct diagnostic approach is crucial for the management of NENs because they have a high clinical and biological heterogenity. In addition IHC is useful in identifying prognostic markers. These tumors were previously suggested to be rare but increasing use of IHC has shown increased incidence and prevalance. With the development of targeted antitumour agents the importance of early diagnosis has been established.

6.2 REVIEW OF LITERATURE

Neuroendocrine neoplasms (NENs) is a heterogenous group of epithelial neoplastic proliferations. They share expression of neuroendocrine markers such as Synaptophysin (intracytoplasmic small presynaptic -like vesicles) and Chromogranin A (large dense core hormone granules) but differ in histologic features, proliferation, hormonal production, molecular profile and clinical aggressiveness.[1,3]

Neuroendocrine neoplasms occur almost everywhere in the body but are most common in GIT, lungs and pancreas. They are mainly of epithelial or neuroectodermal in origin. The epithelial NENs include carcinoid, argentaffinoma, APUDoma, small cell carcinoma, islet cell tumor, neuroendocrine tumor, neuroendocrine carcinoma. The neoplasm with neuroectodermal origin includes paraganglinomas, pheochromocytoma, neuroblastoma and olfactory neuroblastoma. Carcinoid was proposed by Oberndorfer in 1907.He differentiated between cancerous tumors of ileocolic junction and benign behaving lesion (carcinoids) of the ileum in 1907. He defined a series of intestinal epithelial neoplasms which were having a blander morphology.[4]

According to a surveillance report, average incidence of GI NET's is 2.5 cases per 100,000 per year, with a recent increase in United States due to increased detection of gastric and rectal NETs. Gastropancreatic neuroendocrine neoplasms (GEP-NENs) arise from gastrointestinal stem cells which are able to differentiate into neuroendocrine cells.[8] NENs of ileum and appendix were the most common in GEP-NENs but recent studies show gastric NENs as most common. The incidence of GI–NET is around 67.5% amongst all

NETs.They can present sometimes with distinct clinical syndromes such as flushing, diarrhoea, hypoglycemia and gastric ulcers

Neuroendocrine tumors of lung include low grade typical carcinoid (TC), intermediate grade atypical carcinoid (AC), high grade large cell neuroendocrine carcinoma and small cell lung carcinoma. SCLC is the most common lung NENs representing 14% of invasive lung cancer. They are distinguished on the basis of miotic counts, presence and absence of necrosis and cytologic features.[9]

Neuroendocrine breast neoplasia is an uncommon breast cancer. These tumors have a high frequency of estrogen receptor expression.[10]

Neuroendocrine tumors are rare at all sites of female genital tract and often are seen in association with other tumors. In ovary, carcinoid tumors usually arising in dermoid cyst are most common neuroendocrine neoplasms and in uterus high grade neuroendocrine carcinomas are more common.[11]

NENs from different region of body share many morphological and biological features but their classification differs in terminology and stratifying criteria. In 1980 in the first gastropancreatic NETs WHO classification the term carcinoid was used for most of gastropancreatic NETs except for small cell carcinoma and pancreatic islet tumor.

WHO classification 2000 classified NET as well differentiated NETs and poorly differentiated neuroendocrine carcinoma. Well differentiated NETs were further classified as benign and borderline tumors, based on tumor size, miotic rate, proliferative rate, perineural and lymphovascular invasion.[12]

The classifications which are most commonly used are WHO classification for various organs such as –gastropancreatic NETs (GEP–NENs), breast, genital

organs, lung and thymus, urological organs, head and neck, pancreas. Munich classification in 1995 is the backbone for subsequent WHO classification.

The classification that are currently used are the WHO classification of Gastropancreatic NETs (GEP-NENs) 2010 and the 2017 classification covering only pancreatic neuroendocrine neoplasm (PanNENs). The label neuroendocrine neoplasm is used to include all tumors and carcinomas of neuroendocrine cells in these classifications.Thus they divide NETs into 3 groups:

1) Well differentiated NETs -Neuroendocrine Tumors (NETs).

2) Poorly differentiated NETs -Neuroendocrine Carcinoma (NECs)

3) Mixed adeno -neuroendocrine carcinoma

A three tiered grading system based on miotic count and /or Ki67 index is defined by these classifications as-:

G1-(Ki67<3%) and miotic count <2/10 HPF

G2-(Ki67 3-20%) and miotic count 2-20 /10HPF

G3-(Ki67 >20%) and miotic count >20/10 HPF

Imunnohistochemistry (IHC) is a cornerstone in diagnostic and prognostic classification.The minimum panel required for correct classification of NENs includes the general neuroendocrine markers such as Synaptophysin and Chromogranin A and Ki-67 as a proliferation index. Synaptophysin is the most sensitive neuroendocrine marker and Chromogranin A is the most specific.

Majority of these NET tumors have an indolent course. Some of the cases are diagnosed incidentally while some have disseminated disease and can present as metastatic disease.

A retrospective study was done by Amarapurkar et al in which analysis of 74 patients with gastrointestinal and pancreatic neuroendocrine tumors over a period of 7 years was done in Mumbai. The results showed a male preponderance with a mean age of 53 ± 15 years. And out of the 74 tumors, the commonest site was found to be stomach 22, followed by pancreas 17 and duodenum 14. Only 3 patients presented with carcinoid syndrome.[13] Thus concluding that gastrointestinal and pancreatic neuroendocrine tumors were not rare and pattern of these tumors has definitely changed over last few years. Stomach was found to be commonest site for gastrointestinal neuroendocrine tumors followed by pancreas and duodenum.

In another study done by Felipe Silveira for comparing the frequency of neuroendocrine tumors according to age, location, gender and degree of differentiation which was done on 250 patients, lung came out to be the most frequent site followed by stomach and liver metastasis was found in 44 cases.[14]

A study done by Megha S Uppin et al which included 40 cases of GEPNET, most of the tumors were present in small intestine followed by pancreas.[15]

Chromogranin A's usefulness was studied as a marker of neuroendocrine tumors in 238 patients of NETs by Davide Campana et al which confirmed the high specificity and sensitivity of Chromogranin A in diagnosing an NET.[16]

A cancer registry based analysis was done in Denmark on patients who were diagnosed with bronchial neuroendocrine tumor by Halla Skuladottir et al which found that the incidence of neuroendocrine tumors has increased by

two fold among men and by three fold in women, thus concluding that the incidence of neuroendocrine tumors is increasing.[17]

A study was done by Chauhan A to know the global burden of neuroendocrine tumor between 1995 and 2015. It showed an increase in neuroendocrine tumors by 6 folds in United States with an increase in incidence in age group between 50 -64 years.[18]

According to a study done to know the prognostic factors of gastropancreatic neuroendocrine neoplasm at Kaoshiung Chung Gung memorial hospital, 97 patients were included. Large tumor size, non rectal neuroendocrine neoplasm, high histological grading and lymph node metastasis were associated with poor survival.[19]

A survey showed increased incidence of pancreatic neuroendocrine tumors in 2010 by approximately 1.2 times than the cases in 2005 in Japan thus showing the increased incidence of neuroendocrine neoplasm.[20]

6.3 AIMS AND OBJECTIVES OF THE STUDY

To study histopathological spectrum of neuroenocrine tumors.

7.0 MATERIALAND METHODS

All the biopsies and resected specimens of neuroendocrine tumors will be processed and stained with Haematoxylin and Eosin (H&E) stain. The slides will be subjected to histopathological examination under both low power (10X) and high power (40X). Immunostains will be done in all cases and findings will be recorded.

7.1 SOURCE OF DATA

This will be a 3 ½ years retrospective and 1 ½ years prospective study to be conducted in the department of pathology, Dayanand Medical College and Hospital, Ludhiana. All the biopsy and resected specimens diagnosed as neuroendocrine tumors on H&E and immuno histochemistry (IHC) stains will be included in the study.

7.2METHOD OF COLLECTION OF DATA

Inclusion criteria: All the biopsies and resected specimens of primary and secondary IHC proven neuroendocrine tumors.

Exclusion criteria: All tumors which donot show neuroendocrine differentiation by IHC and neuroendocrine hyperplasia.

Methodology: Biopsies and resected specimens will be received in container containing 10% formalin. Tissue will be processed and stained with Heamtoxylin and Eosin (H&E) stain. IHC markers such as Synaptophysin, Chromogranin A, Neuron Specific Enolase will be used.

Duration of study: Retrospective 3 ½ years and prospective 1 ½ years.

Study Design: This is prospective and retrospective study.

Outcome: All the findings of specimen will be documented according to the proforma.

Statistical Analysis: Data will be described in terms of range; mean±standard deviation (± SD), median, frequencies (number of cases) and relative frequencies (percentages) as appropriate. All statistical calculations will be done using SPSS (Statistical Package for the Social Science) SPSS 21 version statistical program for Microsoft Windows.

7.3 DOES THE STUDY REQUIRE ANY INVESTIGATIONS OR INTERVENTIONS TO BE CONDUCTED ON PATIENTS OR OTHER HUMANS OR ANIMALS? IF SO, PLEASE DESCRIBE BRIEF?

None.

7.4 HAS ETHICAL CLEARANCE BEEN OBTAINED FROM YOUR INSTITUTION IN CASE OF 7.3?

Yes.

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PROFORMA

Serial number:

NAME:

Age/Sex:

Father's Name:

MRD number:

Histopath No:

Date

Address:

HISTORY OF PRESENTING ILLNESS:

PERSONAL HISTORY:

Alcohol

Smoking

Others

GENERAL PHYSICAL EXAMINATION:

IMAGING STUDIES:

LABORATORY INVESTIGATIONS

Serum levels of

1)-Histamine

2)-Serotonin

3)-Chromogranin

Signature of Candidate

Signature of Supervisor

HISTOPATHOLOGY:

Biopsy Specimen:

Gross Examination:

Microscopic features :

1-Architecture

2 – Cell Size

3- Nuclear Features

4-Necrosis

5-Mitosis

IHC :

	Intesity 1+,2+,3+	Location N,C,M	Distribution D,F
Chromogranin A			
Synaptophysin			
Neuron specific enolase			
CD 56			
Ki 67			
Others			

N-nucC-cyto,M-memb D-diffuse ,F-focal

FINAL DIAGNOSIS

Signature of Candidate **Signature of Supervisor**